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3rd INTERNATIONAL SYMPOSIUM ON BIOLOGICAL CONTROL OF ARTHROPODS

New Zealand

Proceedings of the
Third International Symposium on Biological Control of Arthropods
Christchurch, New Zealand

February 8-13, 2009

Edited by
Peter G. Mason, David R. Gillespie & Charles Vincent
Agriculture and Agri-Food Canada

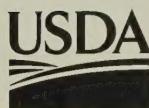
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Peter G. Mason, David R. Gillespie and Charles Vincent (Eds.)

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PREFACE

The Third International Symposium on the Biological Control of Arthropods held in Christchurch, New Zealand builds upon the foundation laid at the first meeting in Hawaii in January 2002 and the second, which was in Davos, Switzerland in 2005. The intent of the ISBCA is to create a meeting for practitioners, a forum for information exchange, an event to build cohesion among the research community, and to foster discussions of issues affecting biological control work, particularly concerning the use of parasitoids and predators as biological control agents.

Biological control has never been more important globally than it is now. Invasive organisms are a major threat to natural and engineered ecosystems with biosecurity measures to mitigate and manage such incursions costing US\$ billions each year. Global warming is likely to produce new pests and change the efficacy of biological control agents. Also, much of current worldwide pesticide use is wasted, insect resistance to insecticides is increasing and with increasingly-discriminating consumers in many countries, pesticide residues in food are increasingly not tolerated, nor are the external costs of pesticide use (damage to human health and the environment). Nearly two billion people worldwide are under-nourished and the prospects for the quality of life of a predicted world population growing to nine billion in a few decades are not good.

Biological control is an ecosystems service (ES) or nature's service and is driven by biodiversity. The latter is declining globally at the fastest rate in the history of humanity and with that decline ES are being lost. "Substitution agriculture", with its dependence on mineral oil for fuel, pesticides and fertilisers is being practised increasingly worldwide to replace lost ES but that is not a sustainable solution. It is vital that we understand more fully the relationship between ES, specifically biological control, and biodiversity, and this is the subject of much current ecological research. Future biological control research must be informed by that scientific debate to minimise the risk of failures in or unexpected consequences of release of biocontrol agents and to understand the ecological mechanisms behind success and failure. It is hoped that this conference has made a significant contribution to moving the science of biological control in those directions for the 21st century.

Steve Wratten, Bio-Protection Research Centre, Lincoln University

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1. The first part of the paper discusses the importance of the study of the history of the United States. It is argued that the study of the history of the United States is essential for a full understanding of the country and its people. The paper then discusses the various methods used by historians to study the past, including the use of primary and secondary sources, and the importance of critical thinking in the study of history.

2. The second part of the paper discusses the role of the federal government in the development of the United States. It is argued that the federal government has played a central role in the development of the country, from the establishment of the government to the present day. The paper then discusses the various policies and programs of the federal government, and the impact of these policies and programs on the development of the country.

3. The third part of the paper discusses the role of the states in the development of the United States. It is argued that the states have played a central role in the development of the country, from the establishment of the government to the present day. The paper then discusses the various policies and programs of the states, and the impact of these policies and programs on the development of the country.

4. The fourth part of the paper discusses the role of the people in the development of the United States. It is argued that the people have played a central role in the development of the country, from the establishment of the government to the present day. The paper then discusses the various policies and programs of the people, and the impact of these policies and programs on the development of the country.

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SESSION 1

NEW AND EMERGING SUCCESSES IN CLASSICAL BIOLOGICAL CONTROL: HAS THEORY IMPROVED PRACTICE?

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This session will examine to what degree some perennially discussed theoretical notions of how classical biological control ought to be conducted have actually guided or improved the conduct of real projects in recent years. This topic is relevant because at bottom, biological control is about doing projects that control pests. Discussions of theoretical issues are useful to expand understanding of mechanisms, but some danger exists that theoretically desirable approaches may continue to be advocated even after they have been discarded by practitioners as unnecessary, misguided or impossible to implement. Therefore the specific objectives of this session are to examine a series of beliefs or assertions that figure prominently in the biological control theoretical literature and see to what degree they are relevant to actual projects. The presentation by Mark Hoddle addresses some predictions made by invasion theory (stratified dispersal and need for minimum population size) in the context of the highly successful control of the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), in French Polynesia. Roy Van Driesche considers whether the oft-advocated task of pre-release prediction of the efficacy of a new natural enemy is feasible, based on studies in the native range, climate matching, agent attributes, or analyses of natural enemy guild structure or pest life tables. This is done in the context of several case histories of real projects. Wade Jenner considers if concerns for protection of non-target species have induced a shift toward introduction of more monophagous agents and if not why not. Max Suckling and Matthew Coll explore the application of new trapping technology (pheromones, sex attractants and kairomones) to biological control as tools to monitor for establishment and test theoretical predictions. Peter McEvoy discusses the use of perturbation analysis of matrix population models to identify how best to intervene in an invading organism's life cycle to change rates of population growth and spatial spread. Finally, Russell Messing discusses how regulations affecting biological control have become more stringent and asks if they are improving the selection of safe natural enemies and increasing the success of classical biological control or are new regulations preventing program development and limiting targets. The expected outcomes of this session will be stimulate discussion of these issues and to guide the further evolution of expectations and policies in ways supportive of the more efficient conduct of safe programs of biological control.

CAN EFFICACY OF NEW BIOLOGICAL CONTROL AGENTS BE PREDICTED BEFORE THEIR RELEASE?

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ABSTRACT.

Pre-release prediction of agent efficacy in classical biological control has long been advocated as desirable but recognized as difficult-to- infeasible. Here we consider this issue noting first reasons why a robust ability to make such predictions would be valuable and then reviewing approaches that have been advocated. Wherever applicable, we contrast approaches for use in weed and insect target projects. We consider the roles of data from the native range, climatic matching, agent attributes, and guild or life table analyses. We examine several predictions made in three insect biological control projects as case histories, to illustrate how actual choices are driven by a mixture of project circumstances, scientist objectives, and the biologies of the agents available in particular projects.

WHY TRY TO FIND “THE BEST” AGENT?

Three reasons have been advanced as to why it may be valuable to identify and release only “the best” agent(s) in classical biocontrol projects. **(1)** The first of these is the possibility that prior introductions of potentially inferior natural enemies may make it harder to establish additional more efficacious species or even lower the total pest suppression achieved because of competition (Turnbull & Chant 1961). Support for this idea comes from either experiments in very simple systems or computer models. In complex landscapes, most evidence suggests that for the majority of programs total pest suppression either stays the same or increases with additional species of natural enemies (Huffaker & Kennett 1969; Hoffmann & Moran 1998). Modeling (Pedersen & Mills 2004) also suggests that most parasitoid combinations are likely to provide additive pest suppression. **(2)** Secondly, it has been suggested that focusing first on agent efficacy can reduce project costs because predicting efficacy will be cheaper than predicting and testing safety (McClay & Balciunas 2005). This argument is largely restricted to weed biocontrol agents and is based on the notion that measuring per capita impacts of agents can predict their efficacy, but this has no application to insect biocontrol projects because attacked hosts or prey typically always die. **(3)** Thirdly, efficacy prediction may reduce risk of indirect nontarget effects, since these occur if agents become abundant but fail to lower the pest density (Holt & Hochberg 2001). However, this perspective overlooks the fact

that in some cases such abundant but not effective agents later prove to be essential components in effective complexes of natural enemies.

OBJECTIVES.

Our objectives are (1) to review the types of information suggested in the literature as predictive of agency efficacy, (2) to contrast the utility of each type of information between weed and insect biological control projects, and (3) to examine several case histories to determine how concepts and events shaped predictions made in particular projects and take note of their accuracy.

DATA FROM THE AGENT'S NATIVE RANGE.

Studies in the native range of the agent offer a number of opportunities to gauge the likely efficacy of a new biocontrol agent through a combination of surveys and experiments (Goolsby *et al.* 2006). Commonly, collectors view agents that are widespread and cause significant damage or mortality to the pest in the native range as the best candidates (Sheppard 2002). To get such information, surveys must quantify pest densities through time, levels of damage from the pest of interest, and the impacts of natural enemies on pest life stages (Hoddle & Hoddle 2008). It is also possible to deploy sentinel groups of pest insects in vulnerable stages to measure mortality from suspected sources like parasitoids of a particular insect stage (e.g., Abera-Kalibata *et al.* 2006). Such studies in the native ranges of pests, however, are not common, in part because of lack of local research facilities, lack of skilled and reliable cooperators, or sufficient time in what are sometimes difficult locations.

CAN CLIMATE MATCHING IDENTIFY THE MOST EFFECTIVE AGENTS?

New agents must be well adapted to the climate of the area where they are released. Programs like CLIMEX and others can match collecting and release locations for a variety of climate variables such as temperature, moisture, photoperiod, cold stress, heat stress, dry stress, wet stress, and the length of the growing season (Zalucki and van Klinken 2006). Collectively these environmental variables affect life history characteristics and can have an important influence on diapause behavior and subsequent survivorship rates. But how well do high climate-similarity index values really predict the efficacy of a species as a biocontrol agent? In one sense, climate matching serves to eliminate some agents that have a poor climate match between their collection and intended release areas. Climatic matching is useful for predicting establishment, which is a requirement for efficacy. Predicting efficacy, however, is more difficult. Climate indices average many aspects of climate, without information as to which parameters matter. To identify limiting factors, detailed studies are needed (e.g., Byrne *et al.* 2002; Gutierrez *et al.* 2008).

DO AGENT ATTRIBUTES PREDICT EFFICACY?

Many authors have commented on the attributes "good" biocontrol agents ought to have (Huffaker *et al.* 1977; May & Hassell 1988; Murdoch *et al.* 2003). However, tradeoffs among life history traits have clearly occurred, and some important attributes such as host finding ability may be difficult to measure in the laboratory. As Mills (2005) notes, real species often combine mixtures of good and bad attributes,

each deviating from the ideal to various degrees. Furthermore, the value of any particular set of attributes is likely to depend on the biology and ecology of the species and the ecosystem into which introduction is intended. Few, if any, general principles have emerged to guide the selection of the 'best' parasitoid species for introduction based on individual attributes. Discussion of attributes commonly of interest follows: (1) High fecundity is easy to measure and has received much attention in the past. However, many examples exist of agents whose introduction was emphasized because of high fecundity and ease of laboratory rearing, yet which failed to control the target pest, e.g., *Pseudoscymnus tsugae* (Sasaji and McClure) (Col.: Coccinellidae) (Cheah & McClure 1998) and *Dahlbominus fuscipennis* (Zett.) (Hym.: Eulophidae) (Lambert 1941). (2) Good host finding ability of insect control agents is often important. Measuring this ability in quarantine, however, is difficult. When attempted, the artificiality of quarantine testing arenas may distort or mask the true host finding capability of an agent. (3) In insect biocontrol, the ability to cause a higher percentage of mortality in patches where pest densities are higher has long been viewed as a fundamental quality of an effective biological control agent (Huffaker *et al.* 1971; Murdoch *et al.* 2003). However, artificiality of test designs prevents meaningful estimation of this parameter in quarantine. Estimates might better be made in the native range, provided that both rates of attack (such as parasitism) and local host density can both be measured at each collection site. (4) The concept of relative length of host and natural enemy generations has been dubbed GTR by Kindlmann & Dixon (1999) and this appears to be a strong predictor of a species' likely success, provided that the natural enemy can attack the host over a broad range of life stages or the susceptible stage occurs over a long period (Mills 2006). GTR values <1 indicate the agent's numbers increase faster than the pest, and species with low GTRs dominate in successful control of scales by parasitoids (Murdoch *et al.* 2006) and coccinellids (Dixon 2000). (5) In contrast, gregarious versus solitary development, rather than low GTR, appears to be a better predictor of success for parasitoid introductions against Lepidoptera (Mills 2006).

CAN GUILD OR LIFE TABLE ANALYSES PREDICT AGENT EFFICACY?

Population models and concepts about how agents or groups of agents affect the target pest have been used to predict what sort of agents might best suppress target pests in biocontrol projects. Here we discuss (1) empty niche/weak link, (2) natural enemy guilds, and (3) population modeling. Detection of "weak links" in the pest's life system has been attempted by comparisons of pest survival in each stage in native vs invaded areas. Briese *et al.* (2002) used this approach in Australia and identified seed reduction as the key goal for biocontrol of *Onopordum* thistles. Mills (2005) compared codling moth (*Cydia pomonella* [L.]) (Lep.: Tortricidae) parasitoid guilds in the native range (Central Asia) to an invaded area (California). Stage-structured models estimate the transition probability for moving through successive stages. These comparisons suggested codling moth population growth would be most reduced in California by adding parasitoids of the second larval instar and of the cocoon stage. Agent selection also included avoidance of species antagonistic to existing parasitoids, such as potential cleptoparasitoids and ecto larval parasitoids that would compete strongly with desirable endoparasitoids. Two of the three parasitoids that were selected did establish and one (*Mastrus ridibundus* [Gravenhorst]) (Hym.: Ichneumonidae) became widespread and reduced the density of the target pest (Mills 2005).

Many weed biocontrol practitioners believe that target plants decline in density only after total stress from various sources exceeds a threshold. For example, to control spotted knapweed (*Centaurea maculosa* Lamarck) (Asterales: Asteraceae), practitioners sought to reduce seed production and increase mortality of established plants. To do this, twelve species, including several seed head flies (Dip.: Tephritidae) and root feeders (e.g., (Col.: Curculionidae) and (Lep.: Tortricidae)) were released (Story 2002). In insect biocontrol, the “cumulative stress theory” was often phrased as a need to “establish a natural enemy complex,” attacking all pest life stages. Early on (1906-1911), the goal of the gypsy moth (*Lymantria dispar* [L.] [Lep.: Lymantriidae]) project in the USA, was to establish as many parasitoids on each of its life stages as possible. In total, over 80 species of parasitoids were released. Only 10 established, and of these only six caused significant mortality (Van Driesche *et al.* 1996).

Models to evaluate natural enemies have largely been for post host impact assessment (e.g., Murdoch *et al.* 2005). Pre-release model building has been rare. Godfray and Waage (1991) built a model during the course of a project to compare two parasitoids (*Gyranusoidea tebygi* Noyes and *Anagyrus mangicola* Noyes [both Hym.: Encyrtidae]) released in Africa against an invasive mealybug. Comparison of parasitoid attributes showed a conflicting pattern, with each species having some advantages (Moore 2004). The model suggested pest suppression would be greatest from *G. tebygi*, which had already been released. It also suggested release of *A. mangicola* would do no harm. *Gyranusoidea tebygi* later became widely established and suppressed the pest (Neuenschwander *et al.* 1994; Bokonon-Ganta & Neuenschwander 1995). *Anagyrus mangicola* contributed to pest suppression mainly in urban areas.

LEARNING FROM CASE HISTORIES.

No single approach to predicting agent efficacy before release is likely to work. Nevertheless, study of case histories of biological control projects in which large agent pools were greatly winnowed can show why some species were not released.

Case 1. *Bemisia tabaci* in the USA and Australia.

Potential natural enemies of the B strain of *Bemisia tabaci* (Gennadius) (Hem.: Aleyrodidae) were collected 1992-1998 via surveys in 19 countries. Eighty collections were sent to quarantine and 38 parasitoid populations of *Encarsia* or *Eretmocerus* were established for study (Goolsby *et al.* 1998). Predators and pathogens were not considered for importation. Many parasitoid populations could not initially be identified but were characterized with molecular tools (Vacek *et al.* 1996), pending identification. Sixteen parasitoid species (Fig. 1) were ultimately recognized (Goolsby *et al.* 2005).

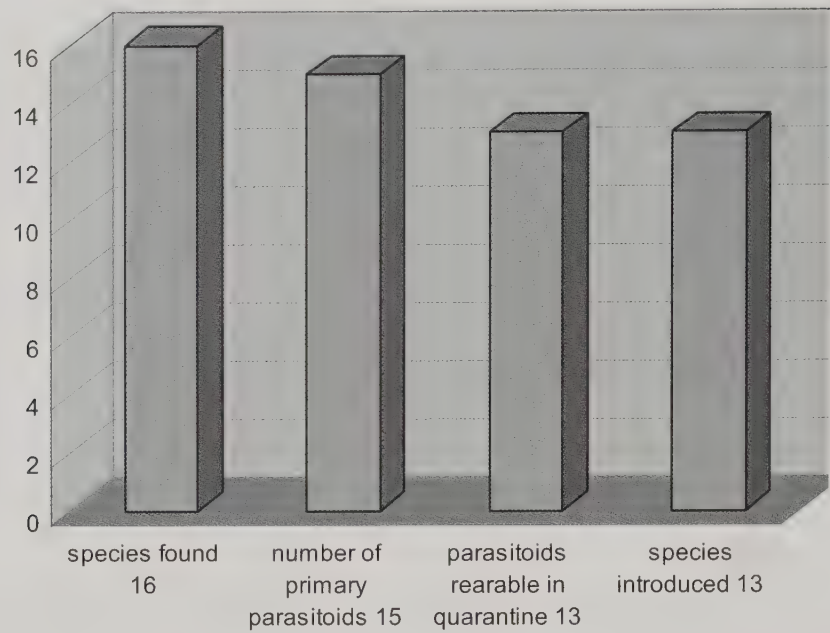


Fig. 1. Against the B strain of *Bemisia tabaci* in the USA, 16 parasitoid species were collected; one was eliminated because of its biology and two because they could not be reared. The other 13 species were released.

Encarsia porteri (Mercet) (Hym.: Aphelinidae) was eliminated because its males attacked lepidopteran eggs. Two primary parasitoids were dropped from consideration because they were difficult to rear. Quarantine and field tests were used to rate likely effectiveness of the parasitoid species, including a progeny production test on key crops in quarantine and a field sleeve cage test (Goolsby *et al.* 1998). Species doing best in these tests were given priority for mass rearing. Quantitative information on rates of parasitism in the native collection areas was recorded (Kirk *et al.* 2001), but this information was not used to pick species for release. All 13 rearable, primary parasitoids obtained from the pest were released. Performance tests later suggested that *Eretmocerus mundus* Mercet and *Eretmocerus hayati* Zolnerowich and Rose (both Hym.: Aphelinidae) were the species most likely to do well and these were prioritized for further mass rearing (Goolsby *et al.* 2005). A post-hoc analysis of climatic similarity and performance identified *E. hayati* as likely to be effective in Australia. This species was released and controlled the pest there (De Barro & Coombs, 2008).

Case 2. Obscure Scale in California.

To control a small infestation of obscure scale (*Melanaspis obscura* [Comstock]) (Hem.: Diaspididae) in Sacramento, California, natural enemies were collected in its native range in the eastern United States (Ehler 1995). Surveys by Ehler on pecan in southern Texas found a total of 12 parasitoids. Literature records (in Ehler 1995) documented attack by five species of mites and two mirid bugs, and infection by three pathogenic fungi, for an initial pool of 22 agents (Fig. 2). Pathogens were excluded because of California's dry climate. Predators were excluded because their host ranges were unknown. Three parasitoids were eliminated as possible hyperparasitoids, four because they were undescribed, and two because they were rare in the native range. This left three candidates: *Coccophagoides fuscipennis*

(Girault), *Encarsia aurantii* (Howard), and *Physcus varicornis* (Howard) (all three Hym.: Aphelinidae).

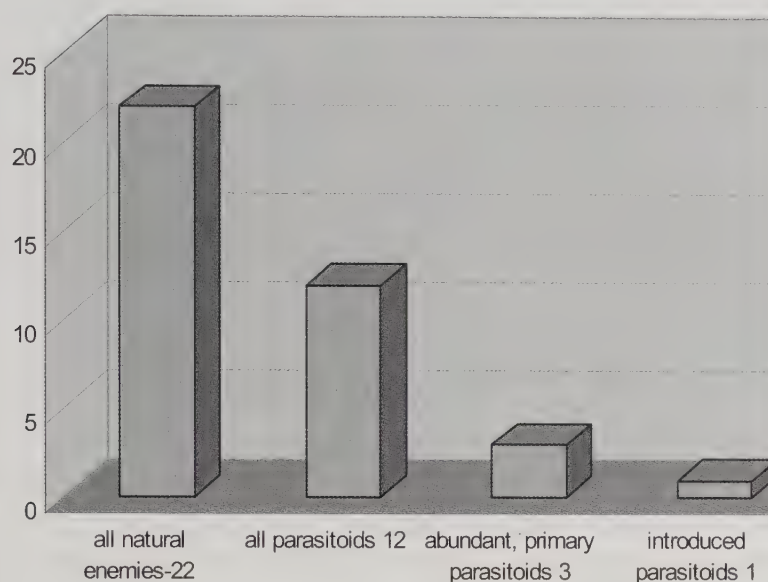


Fig. 2. Narrowing of agent pool for project against the obscure scale: 22 species of natural enemies known to attack the scale (parasitoids, predators, pathogens) in native range surveys plus literature; 12 parasitoid species associated the scale; 3 common, described, primary parasitoids, and 1 species actually released.

Analysis of the life system of the scale in the invaded area was not important in selecting parasitoid species for introduction, as all scale instars were lightly parasitized. Ehler (1985) selected just *E. aurantii* for introduction, even though it had a wider host range than *C. fuscipennis*, because it was thelytokous, had a high egg load, and dominated the parasitoid guild in the collecting area, despite itself being a new association parasitoid for the scale. After its release, obscure scale density declined to desired levels in California (Ehler 2005).

Case 3. Hemlock Woolly Adelgid in the Eastern United States.

Predators in the eastern USA had no measurable impact on the invasive pest, *Adelges tsugae* Annand (Hem.: Adelgidae) (Montgomery & Lyon 1996; Wallace and Hain 2000), prompting surveys in the pest's native range, in four phases: (1) Phase one occurred in Japan (1992) and detected four predators: *Mallada prasina* (Burmeister) (Neur.: Chrysopidae), *Lestodiplosis* sp. (Dip.: Cecidomyiidae), an unidentified syrphid (Diptera), and *Pseudoscymnus tsugae* Sasaji and McClure (Col.: Coccinellidae) (McClure 1995a). Also the mite *Diapterobates humeralis* Hermann (Acari: Orbatidae) was observed feeding on woolly wax of adelgid's ovisacs, dislodging eggs (McClure 1995b). The mite was the most widespread agent, found most frequently, and in greatest abundance. The two most abundant species – the mite and the coccinellid – were imported and released (McClure, 1995b; Cheah and McClure, 1996). The mite, however, was already widely present in the USA, being an omnivore able to feed on lichens. Neither agent reduced the pest density, nor has any prospect of doing so. (2) Phase two (1995/1997-present) occurred in China, where 54 ladybird species were found on hemlocks infested with adelgids (Yu *et al.*

2000). *Scymnus sinuanodulus* Yu and Yao, *Scymnus camptodromus* Yu and Liu, and *Scymnus ningshanensis* Yu et Yao (all three, Col.: Coccinellidae) were the most abundant, seemed to be adelgid specialists, and accordingly were imported to US quarantine for study. The first two species have been released, but *S. camptodromus*, considered the best candidate, is still in quarantine because an egg diapause has made it difficult to rear. Neither species has yet reduced the pest's density. (3) Phase three (1997-present) was the importation of the derodontid beetle *Laricobius nigrinus* Fender (Col.: Derodontidae) from Victoria, Canada (and later, a separate population from northern Idaho). Following study of its host synchronization and life history (Zilahi-Balogh *et al.* 2003), and host range, *L. nigrinus* was widely released and is established in 25 or more locations (Mausel 2007), excluding the most northern latitude and highest altitude sites. (4) Phase four (2005-present) surveyed locations in China, Japan, and the northwestern United States. Four new Asian *Laricobius* species were discovered and *Laricobius kangdingensis* Zilahi-Balogh and Jelinek (China) (Col.: Derodontidae) (Zilahi-Balogh *et al.*, 2007) and *Laricobius* sp. (Japan) were shipped to quarantine, as well as *Tetraphleps galchanoides* Ghauri (Hem.: Anthocoridae), found in China on high density adelgid populations. Surveys in Oregon and Washington found 55 predator species associated with the pest, of which *L. nigrinus* and the flies *Leucopis argenticollis* Zetterstedt and *L. artifiaces* (Aldrich) (both Dip.: Chamaemyiidae) were most abundant. In Japan, generalist cantharids were abundant on high density, spring adelgid populations but were not importated. In China, melyrid beetles and large lady beetles (subfamily Coccinellinae) likewise were abundant on high density fall adelgid populations, but were not imported. The chamaemyiid flies from western North America, while specific, could not be reared in the laboratory, and so have not been imported to quarantine.

In total, of 65 potential agents detected in surveys, five have been released (Fig. 3), but only one (*L. nigrinis*) has established and spread. Apart from work in phase one, choice of agents for release has been based on (1) specialized feeding habits that are well focused on adelgids and not a wider range of Hemiptera, and (2) synchronization to *A. tsugae*, with summer aestivation to allow survival when adelgids are not available (Montgomery *et al.* 1998; Zilahi-Balogh *et al.* 2003). Assumptions or implied predictions of the project have been: (1) generalist predators help reduce occasional adelgid outbreaks in Asia but are not suitable for introduction; (2) *Laricobius* spp. have the highest probability of being effective; (3) *Leucopis* spp. have potential but are limited by difficulties of identification and rearing; (4) Idaho *L. nigrinus* will establish better than Seattle *L. nigrinus* in cold areas; and (5) *S. sinuanodulus* will match the climate of the southern Appalachians and *S. ningshanensis* that of New England. Prediction 1 is clearly true and nothing occurred to change the perspective that specialized agents are required. As suggested by prediction 2, *L. nigrinus* has established readily and is spreading (Mausel 2007). Predictions 3 and 5 need additional testing. Supporting prediction 4, Idaho *L. nigrinus* were more tolerant of low winter temperatures than Seattle beetles (Mausel, pers. comm.).

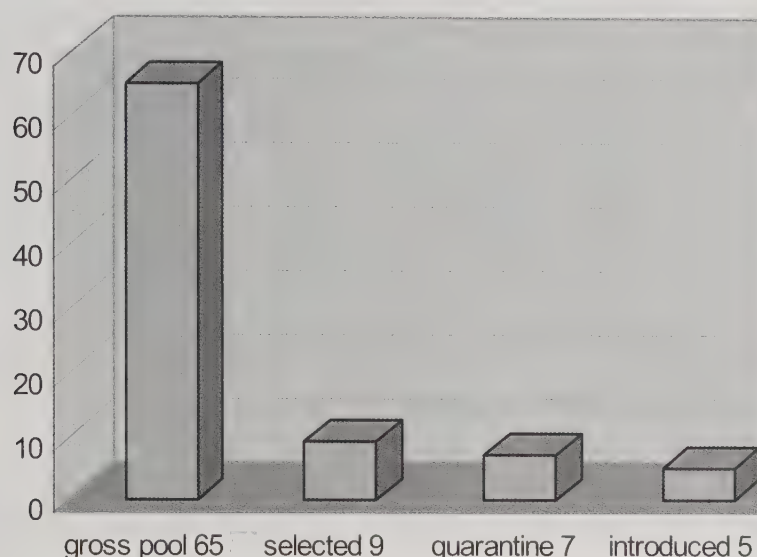


Fig. 3. Numbers of candidate species at various stages of consideration for control of hemlock woolly adelgid, *Adelges tsugae*, for all phases pooled, excluding the generalist predators of the western USA.

CONCLUSIONS.

Per capita efficacy of agents and high climatic match indices are currently emphasized by some weed biological control practitioners but per capita efficacy is unique to weed agents and has no applicability to insect agents. A good climatic match between agents and climates of the receiving region is a necessary but not sufficient condition for efficacy and so is predictive mostly in the negative. Agent attributes can be predictive but are most so within well defined groups of agents and hosts, such as scales and their parasitoids. More broadly, this approach breaks down because attributes often do not integrate into “winning combinations” that exist in recognizable form in actual species. Guild or life table analyses has explanatory value but may not be available for many projects at the point when decisions about which agents to take into quarantine for study as actually made. Close examination of many case histories, with special attention to predictions made and their accuracy is advocated as useful. Efficacy prediction is not currently possible, but attempting to make such predictions in the course of biological control projects is desirable as a means to improve ability to do so.

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CAN INVASION BIOLOGY THEORY ASSIST CLASSICAL BIOLOGICAL CONTROL PROGRAMS?

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ABSTRACT.

The classical biological control program against an invasive pest in French Polynesia with an exotic parasitoid provided an ideal situation to examine factors affecting the invasion success of a deliberately introduced organism. Theory from Invasion Biology predicts that invasion success will be affected by: (1) the invader's pattern of dispersal and, (2) resource abundance in habitats into which the invader penetrates. In the study reported here, the invasion success of a parasitoid was promoted by its ability to undergo exponential dispersion via stratified dispersal. Upon entering new habitats, establishment was affected by resource abundance, and a critical minimum density of hosts was needed for the parasitoid to establish. However, this minimum host density was not a barrier to establishment once parasitoid propagule density crossed an undetermined threshold.

INTRODUCTION.

Releases of arthropod natural enemies as part of classical biological programs are carefully orchestrated invasions that are executed to maximize the likelihood of an exotic organism establishing and then spreading rapidly throughout the range occupied by the target pest. The classical biological control program against the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) in French Polynesia with the mymarid egg parasitoid, *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae), provided an excellent opportunity to study factors affecting the invasion outcomes of this natural enemy on the target pest from the time of initial release on the island of Tahiti. Field survey results in French Polynesia as part of this biological control program have been studied within the context of monitoring an "invasion" to gain insight as to what factors theory suggests should promote the successful invasion of a natural enemy when released as a classical biological control agent.

Homalodisca vitripennis is native to the southeastern U.S.A. and northeastern Mexico. Females lay their eggs within plant tissue, usually on the undersides of leaves of shrubs and trees. The adults and the five nymphal stages are xylophagous and extremely polyphagous, having been recorded feeding on more than 150 plant species in 34 plant families. *Homalodisca vitripennis* invaded Tahiti, French Polynesia in 1999 (Grandgirard *et al.* 2006; 2008). It reproduced and spread very rapidly in French Polynesia and is currently found throughout the Society Island archipelago and has spread to Nuku Hiva in the Marquesas archipelago (1,400 km

northeast of Tahiti) and to Tubuai and Rurutu (600 km south of Tahiti) in the Australs archipelago (Grandgirard *et al.* 2006; 2008; Petit *et al.* 2008).

In French Polynesia, large populations of *H. vitripennis* caused numerous problems. Firstly, it was a major public nuisance because massive densities of feeding adults and nymphs generated high quantities of watery excreta that literally rained from trees (hence its common French name 'mouche pisseuse', the pissing fly). In addition to rain, large numbers of *H. vitripennis* would fly into houses and businesses at night attracted to lights. Secondly, continuous year round feeding by high populations was suspected of retarding plant growth and causing declines in fruit production. Thirdly, *H. vitripennis* as a vector of *Xylella fastidiosa* Wells *et al.* (Xanthomonadales: Xanthomonadaceae) the causative agent of Pierce's Disease, a lethal plant malady, was a potential major threat for Polynesian plants (agricultural, ornamental, and native). This xylem-dwelling bacterium has not been recorded in French Polynesia, but it could arrive in plants imported into Tahiti from the Americas where the bacterium is endemic. Finally, the immense population densities in French Polynesia made it hard to contain this pest at ports and airports. Numerous live individuals and eggs on plants were found in boats and planes moving between islands. Consequently, *H. vitripennis* invaded Easter Island in 2005, and the Cook Islands in 2007, most likely originating from Tahiti as eggs transported on ornamental plants. Thus French Polynesia represented a major invasion threat to neighboring island countries and trading partners.

To mitigate all of these problems, a classical biological control program against *H. vitripennis* using *G. ashmeadi* was initiated in French Polynesia. *Gonatocerus ashmeadi* is a solitary endoparasitoid attacking eggs of Proconiini sharpshooters (Cicadellidae: Cicadellinae: Proconiini). In its native range, southeastern U.S.A. and northeastern Mexico, *G. ashmeadi* is a common and effective parasitoid of *H. vitripennis* eggs. This parasitoid is self-introduced into California where it is the dominant natural enemy attacking *H. vitripennis*. *Gonatocerus ashmeadi* was imported into quarantine in Tahiti from California in September 2004, and after non-target impact assessments were completed official releases into the environment commenced in May 2005. A total of 13,786 parasitoids were released at 27 sites on Tahiti between May and October 2005. Within seven months of release, *G. ashmeadi* had reduced pest populations on Tahiti by more than 95% and parasitism rates of *H. vitripennis* eggs were averaging 80-100%. Control of *H. vitripennis* was fast and efficient in part because of the extremely rapid and widespread dispersal of *G. ashmeadi*. Of interest here is an understanding of the factors that promoted the successful invasion of *G. ashmeadi* throughout French Polynesia.

FACTORS AFFECTING THE DISPERSAL OF INVASIVE ORGANISMS.

The dispersal characteristics of natural enemies (and invasive species in general) have a strong effect on their invasion success. Natural enemies that demonstrate high invasion potential are likely to adversely affect pest population growth if the entire range of the pest is infiltrated and colonized rapidly. The rate of spread of a natural enemy will be strongly influenced by host abundance, and pest densities will in turn be affected by the availability of resources needed for population growth (Petit *et al.* 2008). Understanding the interplay of these different factors (i.e., rate of spread and host abundance on the likelihood of natural enemy establishment) on the dispersion of an invasive organism is an important applied problem in Invasion

Biology. Consequently, monitoring the movement of *G. ashmeadi* in an ecosystem comprised of islands and widely separated groups of islands provided a unique opportunity to monitor the spread of an “invasive species” in a new ecosystem from the exact moment of its introduction. Following release on Tahiti (the only island in French Polynesia on which this parasitoid was released), *G. ashmeadi* dispersal was monitored to determine its dispersal characteristics on and between islands, and the effect host density in different ecosystems (e.g., urban and natural areas) had on parasitoid establishment.

Parasitoid Dispersal Characteristics.

It is not uncommon for invasive insects to display stratified dispersal once populations are established in a new area. Stratified dispersal is a combination of: (1) short distance spread either by flying or walking, and (2) rapid long distance dispersal that may be mediated by the wind or unintentional movement by humans (Petit *et al.* 2008). The island system of French Polynesia made it very easy to study the dispersal mechanisms for *G. ashmeadi* because short-distance dispersal across an island from known release sites was easily separable from long-distance movement of this parasitoid between islands and archipelagoes.

Short Distance Dispersal of *G. ashmeadi*.

On Tahiti, monitoring of *G. ashmeadi* dispersal via the collection of *H. vitripennis* egg masses and subsequent rearing of parasitoids indicated that this parasitoid was moving on average at a rate of 47 m/day along the coast where pest populations were very high (~156 *H. vitripennis* nymphs collected per 1 minute of sweep netting hibiscus) and the flora was a rich mixture of exotic ornamental plants in urban residences. Dispersal was equivalent in easterly and westerly directions indicating that prevailing sea winds did not influence parasitoid spread in any single direction on Tahiti (Petit *et al.* 2008).

Long Distance Dispersal of *G. ashmeadi*.

The long distance spread of *G. ashmeadi* from Tahiti to other islands and island archipelagoes was determined by monitoring for the presence of *G. ashmeadi* on Moorea: (Windward Islands 17 kms from Tahiti); Huahine, Bora Bora, Raiatea, Tahaa, and Maupiti in the Leeward Islands (~200-300 km west of Tahiti); Nuku Hiva in the Marquesas Islands (~1,400 km northeast of Tahiti); and Rurutu and Tubai in the Australs archipelago (~600 km south of Tahiti). *Gonatocerus ashmeadi* was not released on any of these surveyed islands and previous surveys monitoring the distribution of *H. vitripennis* indicated that this parasitoid was not present prior to release on Tahiti. The parasitoid was found on Moorea in September 2005, four months after the initial release on Tahiti in May 2005. *G. ashmeadi* was found throughout the Leeward Islands 4.5 to 7.5 months after the initial release in Tahiti. Parasitized *H. vitripennis* egg masses were detected in the Marquesas and Australs archipelagoes 7.5 and 8.5 months, respectively, after the release of *G. ashmeadi* in Tahiti. *G. ashmeadi* was almost certainly moved long distances from Tahiti as parasitized eggs on plants being moved between islands.

DENSITIES OF *H. VITRIENNIS* REQUIRED FOR ESTABLISHMENT OF *G. ASHMEADI*.

The density and distribution patterns of *H. vitripennis* had a strong influence on the invasion and establishment success of *G. ashmeadi*. Parasitoid arrival in areas with very high *H. vitripennis* densities (~150 nymphs/minute of sweep net sampling in coastal urban areas on Tahiti) resulted in immediate establishment because of abundant host eggs being available for parasitism. In high altitude areas dominated by native vegetation where host density was low (<2 *H. vitripennis* nymphs/minute of sweep net sampling) parasitoid establishment was non-existent despite repeated and regular releases of parasitoids into this type of habitat on Tahiti. In this instance, lack of establishment may have resulted because host eggs were too rare and too widely dispersed for parasitoids to locate. Mate finding following emergence from *H. vitripennis* eggs in low host density environments was unlikely to have been a problem because *G. ashmeadi* readily engages in sib-mating upon emergence from egg masses.

However, in high altitude natural areas where *H. vitripennis* densities were typically too low to allow parasitoid populations to establish following deliberate releases, *G. ashmeadi* did eventually establish ~100 days after initial releases at low elevation coastal sites on Tahiti. It is thought that parasitoid establishment occurred because huge numbers of *G. ashmeadi* were naturally invading these high altitude natural areas from coastal areas which supported very high populations of *H. vitripennis* and parasitoids. The sheer numbers of parasitoids working their way up into the mountainous interior regions of Tahiti likely overcame establishment barriers that deliberate releases were unable to do.

Self-introduced populations of *G. ashmeadi* onto islands other than Tahiti readily established when *H. vitripennis* densities were at least ~7 nymphs/minute of sweep net sampling. It would therefore appear that a critical host density is required for the self-establishment of *G. ashmeadi*.

CONCLUSIONS.

The classical biological control of *H. vitripennis* by *G. ashmeadi* was affected by at least two critical factors that theory from Invasion Biology suggests affects the invasiveness of exotic species in new areas. First, the pattern of dispersal has a strong effect on the rate of spread of an invasive organism. In this study, *G. ashmeadi* exhibited stratified dispersal which was characterized by fast short distance spread from release sites (~ 47 m/day) and this was complemented with rapid dispersion over vast distances via the human-assisted movement of plant material bearing parasitized *H. vitripennis* eggs. Exponential spread via stratified dispersal as observed for *G. ashmeadi* in French Polynesia certainly resulted in this exotic organism spreading further and faster than would be predicted by simple diffusion from point sources, thus making *G. ashmeadi* an extremely successful invasive species. In fact, within 10 months of release on Tahiti, *G. ashmeadi* had invaded and established on every island in French Polynesia infested with *H. vitripennis*. In contrast, it took *H. vitripennis* ~six years to achieve the same distribution in French Polynesia. The second factor, as predicted by theory, affecting establishment of *G. ashmeadi* was host abundance in areas in which the parasitoid infiltrated. In habitats characterized by < 2 *H. vitripennis* nymphs/minute of sweep net

sampling *G. ashmeadi* failed to establish despite regular releases into these areas. In contrast, parasitoid establishment was immediate in habitats with >150 nymphs/minute of sampling. In areas where *G. ashmeadi* established without human assistance host densities were around 5-7 nymphs/minute of sweep net sampling which indicated these host densities were adequate for founders to establish. Understanding the relationship between host abundance and parasitoid establishment is very important for designing and planning release strategies to both promote the invasion success of *G. ashmeadi* and to maximize cost effectiveness of the biological control program. Many releases of large numbers of *G. ashmeadi* will be needed in areas with < 5-7 nymphs/minute of sampling to increase the likelihood of establishment. Conversely, few small releases of this parasitoid will be needed when sampling indicates that host densities are >5-7 nymphs/minute of sampling.

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CLASSICAL BIOLOGICAL CONTROL IN A NEW REGULATORY ENVIRONMENT

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ABSTRACT.

Biological control regulations have become much more stringent in recent years. This has led to increasing levels of scrutiny for newly imported biocontrol agents. Is this scrutiny improving the selection of natural enemies, enhancing safety, and increasing the success rates of classical biological control? Or are tougher regulations preventing program development, and limiting targets because of high costs and bureaucratic obstacles? Ecological theory helps to guide the development and implementation of arthropod importation and release protocols, but public (and academic) perception of risk is often driven by factors more sociological than ecological.

INTRODUCTION.

The introduction of exotic natural enemies to control invasive pests has become more tightly regulated in recent years in several countries around the world. This is largely in response to a shift in social standards. Society in general has become more risk averse, and greater value is currently being placed on native ecosystems, biological diversity, and endemic species than in the past. Also, the environmental and economic costs of invasive species have become more widely appreciated. As the regulation of classical biological control becomes more stringent, an increased level of ecological, legal, and bureaucratic oversight of individual projects is leading to changes in the way biocontrol is conducted.

This is especially true for classical biological control of arthropods. The biological control of weeds has a longer track record of non-target testing and risk analysis, due to the greater perceived value of economically important plants that might be at risk from newly introduced herbivores. Weed biocontrol, while also having to adjust to a new regulatory environment, has a head start in the development of protocols for risk analysis, and a more sophisticated and evolved regulatory structure, at least in some countries.

Resources (time, money, knowledge) have always been a limiting factor in the success, and even in the initiation of new biological control projects. As each new natural enemy introduction demands more resources to clear regulatory hurdles, how is it affecting the discipline across the board? Is increased knowledge of the biology of each natural enemy leading to an increased success rate in establishment and control of target pests? Is the greater level of host-range testing leading to improved safety for new introductions? Are increased costs to obtain permits for each project

draining a limited pool of resources, and leading to a decline in the rate of program development? Can responses to emergency situations be conducted in a timely manner?

REGULATORY FRAMEWORKS.

While answers to at least some of these questions may seem apparent, there are scant data to weigh in on any of the hypothetical effects. Australia, Canada, New Zealand, South Africa, the United States, and the State of Hawaii within the U.S. have all tightened their regulatory requirements for classical biological control in recent years (and to a lesser extent Europe, though with more emphasis on augmentative biocontrol). However, the circumstances in each country differ greatly, both in historical context, and in the extent and manner in which new regulations have been implemented. Time frames and economies also differ, as do the emphasis on weed vs. arthropods as targets. Many of the biocontrol projects carried out under these new regulations are still in the early stages of analysis. Therefore, an evaluation of the impact of tightened regulations on biocontrol implementation and success can only be preliminary at this point, and largely anecdotal.

An excellent review of the biocontrol regulatory frameworks for these countries is presented in Sheppard *et al.* (2003). While some changes have occurred in the five years since this publication, the paper thoroughly sets the stage, especially for weed control, and rightly asks the question if there is a “*crisis in the making*,” where new approaches to risk analysis “*run a high risk of grinding biological control releases to a halt*.” A more recent review (Hunt *et al.* 2007) further highlights the strengths and weaknesses of divergent administrative approaches to regulating biocontrol. Recent communications from colleagues around the world reflect diverse perceptions about the impacts of new regulations on the practice of classical biocontrol in each country. This is to be expected in a rapidly changing matrix of backgrounds, project goals, and bureaucratic cultures.

There’s been no apparent reduction in the number of applications for biocontrol permits in New Zealand under the Hazardous Substances and New Organisms Act of 1996. About 17 permit requests for new natural enemies (for 11 target species: 8 weeds and 3 arthropods) have been processed in the past 10 years, a rate similar to that in the decade prior to the new regulations. Those familiar with the system state that “*the pace of weed agent introductions has not been slowed by the Environmental Risk Management Authority process, but there has been a real paralysis in the introduction of insect control agents*.” This has been attributed to apprehension among researchers that the potential non-target effects of parasitoids cannot be adequately predicted. It is too soon to say whether the relative rates of success of biocontrol differ for those agents brought in before vs. after the new law. In terms of safety, there were very few measurable non-target impacts before the change, so it will be difficult to determine if the new process has in any way improved environmental safety.

The New Zealand approach to risk/benefit analysis for biocontrol introductions is arguably the most comprehensive and transparent in the world. However, this thorough analysis comes at a cost – with each applicant required to pay as much as

USD\$90,000 for a review. A high cost such as this is likely to eliminate the start-up of new projects that could be useful to smaller crops, or to natural ecosystems, where less research funding may be available. For example, the successful biological control of the filbert aphid, *Myzocallis coryli* (Goetze) (Homoptera: Aphididae), in hazelnut orchards of Oregon, USA (leading to large reductions in pesticide use, and cost savings for growers of ca. USD\$2,000,000. annually) was accomplished by a single graduate student with a total operating budget of less than USD\$20,000 (1985 dollars) (AliNiazee 1998). It is very unlikely that this project would ever have been started had such high review costs been known at the outset.

Biocontrol in Australia and South Africa appears to have been impacted the most by the new regulatory environment. It's been reported that permits in Australia "*are now grinding to a halt as non-specialist people in key positions fail to take informed decisions.*" In the past 10 years, CSIRO has had only one entomophagous agent cleared through the review system, though this it is not necessarily a result of regulatory obstacles. Rather, it may have to do with the overall cost of projects (the introduction and evaluation of the whitefly parasitoid *Eretmocerus hayati* Zolnerowich & Rose (Hymenoptera: Aphelinidae), for example, reportedly cost AUD\$2.3 million). In South Africa, researchers report that new biocontrol legislation and oversight by the Dept. of Environmental Affairs and Tourism "*has crippled weed biocontrol,*" with permits now required from two separate government agencies, and no noticeable improvement in safety over the previous peer-review procedure.

In North America, the requirements set out in the North American Plant Protection Organization (NAPPO) guidelines (NAPPO 2006) attempt to harmonize the information that must be provided to regulatory officials in Canada, Mexico and the United States when a submission for release is made. This standard format facilitates submission for release of biological control agents to all three NAPPO countries and to provide timely review. This is particularly important where an agent is proposed for simultaneous release in more than one country.

In Canada, new biocontrol regulations have been in place for only 8 years, during which time there were nine applications for release submitted for entomophagous agents (six of which were approved). Submissions are peer-reviewed, and the turn-around-time from submission to decision takes 6 months. Again, it is too soon to evaluate rates of establishment or control based on these six agents alone. The time required to implement new projects has reportedly increased, as have costs, but these are not well quantified.

In the United States, federal regulations for biocontrol have not, in fact, changed substantially in recent years, except for the requirement of an Environmental Assessment to satisfy NEPA (the National Environmental Policy Act, a law passed in 1970 but not enforced in this respect until 1992). Nevertheless, there is a broad consensus (acknowledged by APHIS, the regulatory agency), that the permitting process has not been operating efficiently, and is in need of revision. The Plant Pest Control Act of 2000 gave APHIS explicit authority to regulate all introduced biocontrol agents, and, in response to stakeholder feedback, APHIS is currently in the process of assessing and revising its regulatory guidelines (Title 7 CFR parts 330.200-212).

There have been some procedural changes in the U.S.: biocontrol permit applications for releases are currently required to conform to the NAPPO guidelines,

and these are now routinely reviewed by authorities in Canada and Mexico, in addition to U.S. officials. Restrictions that were put in place following the terrorist incidents of 2001 (i.e., a prohibition against hand-carrying biocontrol agents into the country from abroad; a policy mandating use of licensed bonded commercial carriers) have now been rescinded.

The State of Hawaii has a separate, and much stricter regulatory system for the introduction of biological control organisms than the other 49 United States. The impact of these stringent regulations (and their inefficient implementation) has been previously documented (Messing & Purcell 2001). While there are concurrent forces at work (i.e., declining agricultural economies) in Hawaii, there is little doubt that an increased level of biological, legal, and bureaucratic oversight is a major factor in the precipitous decline of biocontrol introductions shown in Fig. 1.

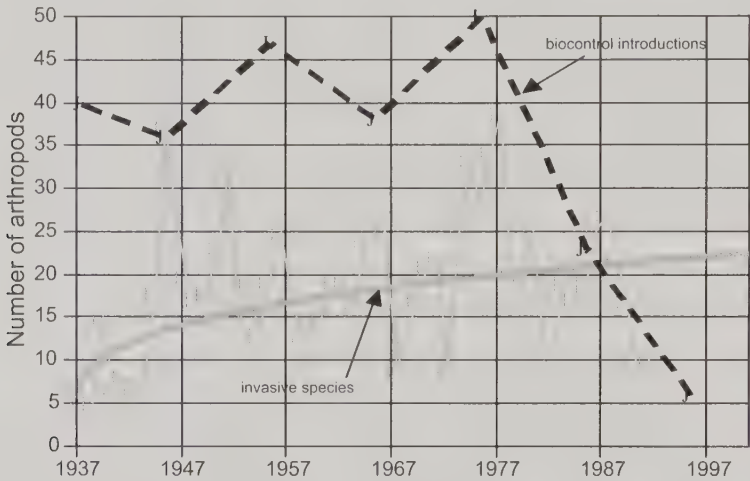


Fig. 1. Number of biological control introductions, by decade, and rates of invasive species incursions into Hawaii, 1937-1997 (data from Hawaii Dept. of Agriculture).

As of this writing (October 2008), an even more extensive review procedure than previously required for introductions of biocontrol agents in Hawaii is being enforced under the Hawaii Environmental Policy Act (HEPA). While requiring much the same information already required by existing Hawaii Dept. of Agriculture (HDOA) procedures, the State is demanding an Office of Environmental Quality Control Environmental Assessment *prior to* the review by the HDOA sub-committees, committees, and board. In other words, simultaneous review by several disparate State agencies is disallowed, and thus an additional 6 months or more may be added to the review process. Furthermore, this new requirement is also being applied to previously-granted permits that come up for a simple renewal.

Much has been written about the merits of more or less stringent permitting requirements for the importation of new natural enemies (Louda et al. 2003; Messing 2000; Messing & Purcell 2001; Sheppard *et al.* 2003; Simberloff & Stiling 1996; van Driesche & Hoddle 1997). Several authors have proposed an approach that relies more heavily on the “Precautionary Principle” (McEvoy & Coombs 2000; Simberloff 2005). However, as pointed out by McCoy & Frank (*in review*), “the precautionary principle applied to biological control falls short as a guide, because it does not

provide a prescription for action.” In the face of uncertainty, and with no prescription for action, decision-making can slow to a halt. In the U.S. system, in Hawaii, and reportedly in Australia and South Africa, uncertainty in the risk analysis process has led to some interminable delays and slow grinding bureaucracies: it is not so much that permits are denied, but rather decisions simply are not made. New Zealand, in contrast, recognizes this potential pitfall and has built fixed timelines into their process, such that decisions must generally be reached within 100 working days.

Failure by officials in a regulatory capacity to act in a timely manner can lead to negative environmental impacts that are every bit as serious as the consequences they are meant to forestall. For example, recent invasions of Guam by the Asian cycad scale, *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae), and of Hawaii by the Erythrina gall wasp, *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae), have threatened to cause species extinctions and major ecosystem disruptions. With biocontrol the only realistic method of controlling these invasive pests, the timely issuance of permits becomes a key link in a successful response strategy. Failing to issue a permit in a situation such as this may be analogous to failure to officially list an Endangered Species by the appropriate government agencies, and may be subject to similar lawsuits. That this has not yet occurred is probably due to the fact that biocontrol has historically been focused on agroecosystems; but this is changing rapidly, as more and more environmental pests become targets of biocontrol (Hoddle 2004).

FADS IN ECOLOGY.

As mentioned in the opening paragraph of this paper, regulations are implemented by government agencies in response to changing public perceptions, judgements, and values. The theme of this symposium is to focus on how theory has improved the practice of biocontrol – so we might question whether risk analyses, decision-making, and the issuance of permits are informed by ecological theory, or perhaps by other forces. The amount of risk tolerated by the public (and, in turn, by its elected representatives and appointed regulatory authorities) is not static. Risk aversion is higher today than it has ever been in relation to classical biological control. But to what extent is this aversion based on theory, or even on the experimental and empirical record?

Ecological theory can tell us, in general terms, which situations lead to more or less risk of non-target impacts (Holt & Hochberg 2001). This can justifiably be used as a rationale for avoiding the “lottery model” of multiple species introductions (McEvoy & Coombs 2000). However, for any individual project, we are still a long way from being able to quantify with any degree of accuracy the overall risk of direct and (especially) indirect effects to non-target organisms. This makes it correspondingly difficult to choose the “best” natural enemy, if several are available. Even were it feasible to choose the best biocontrol agent to introduce based on efficacy against the target organism (see Van Driesche, this volume), *“the ability to predict indirect effects, given that the quantification even of direct interactions is so intractable, seems a long way off”* (Lonsdale *et al.* 2001). This point has been affirmed by other basic and applied ecologists (Holt & Hochberg 2001; Stiling & Simberloff 2000).

Clearly, changes in countries around the world leading to more stringent biocontrol regulations are not the result of dramatic new developments in ecological theory. Rather, several retrospective analyses of individual projects (Louda *et al.* 2003; Boettner *et al.* 2000) and some broader reviews of biocontrol databases (Lynch & Thomas 2000; Hawkins & Marino 1997) have documented real problems in a handful of systems that garnered a great deal of attention among applied ecologists and the general public. This took place in the societal context of a general awakening to the environmental and economic dangers of exotic, invasive species – as well as fierce controversies surrounding the field release of genetically modified organisms.

It may be informative to look at some widely cited examples in the recent literature that report on biological control “impacts,” in order to help evaluate to what extent this widespread perception of risk is based on careful science.

In a paper titled “Infiltration of a Hawaiian Community by Introduced Biological Control Agents,” Henneman & Memmot (2001) stated that: “*the level of attack by alien parasitoids [on endemic Hawaiian Lepidoptera] is estimated to be 19% in 1999, and 22% in 2000.*” This paper has been widely quoted as an example of biocontrol gone awry: it has been cited over 100 times in the scientific literature, inspiring comments such as: “*studies report significant ecological effects on native species of insects*” (Louda *et al.* 2003); “*objective field studies are now confirming that there have been significant non-target effects following some biological control introductions*” (Kairo *et al.* 2003); and “*The finding that most of the parasitoids reared from native Hawaiian Lepidoptera were imported as biological control agents shocked many in the ecological and entomological worlds*” (Stiling 2004).

Then there is a snowballing effect: the Stiling (2004) paper which cites Henneman & Memmot (2001) is itself cited by at least 13 other papers (Google Scholar, accessed October 2008), and some of those papers have in turn been cited numerous times, and so on. Thus does an idea become firmly entrenched in the literature. But is the original estimation of non-target impact on Hawaiian Lepidoptera accurate? And are the conclusions of the paper valid?

Henneman & Memmot (2001) began their study by collecting all caterpillars in two high elevation study sites on Kauai, but those in the genus *Hyposmocoma* were subsequently dropped from their analysis - because that genus was attacked only by native parasitoids, and thus were unlikely to reveal any non-target effects. Thus, the samples that form the core of the paper were pre-disposed to a given conclusion from the outset.

The authors further state that their study sites were isolated geographically, altitudinally, climatically, and ecologically from agricultural areas – (leading Reuters news service to describe the sites as “*really remote*”). Yet commercial sugarcane plantations, with their associated pests and natural enemies, were grown at 700 m in elevation along a major roadway less than 10 miles from the study sites. With both onshore (Kona) winds and large volumes of tourist traffic spanning this distance frequently, it is not surprising that these small parasitoids could be transported to the new environment (see also Hoddle, this volume).

Furthermore, while it may be true that the study sites are somewhat of “*an extreme environment for Hawaii*” (Stokstad 2001), the more apt comparison is to the

alien parasitoids' region of origin (Texas), which almost surely has greater temperature extremes than the Hawaiian study sites. While biocontrol theory encourages climate matching as a means of increasing efficacy and possibly limiting unwanted dispersal (see Goolsby, this volume), it is not only the absolute difference in climate that is important, but also the direction of variation (*i.e.*, it is easier for an arthropod from a harsh mainland climate to survive and thrive in Hawaii than vice versa).

Parasitoids from the only two genera (*Cotesia* and *Meteorus*) that were frequently reared from native Lepidoptera are well known to induce developmental arrest and/or delayed development in their hosts (Shi *et al.* 2002); thus, beating sheet samples at regular intervals are statistically more likely to obtain parasitized than unparasitized hosts of any given cohort. Finally, percent parasitism (as presented in the paper) in no way represents the "indispensable mortality" that is the true measure of population impact (Bellows *et al.* 1992) – sampled individuals were removed from the field and no longer exposed to predators, diseases, and other sources of mortality.

A cascading series of biased steps was built into this study, all of which magnified the perceived impact of parasitoids on native insects. While the authors acknowledge that "*there are clearly important environmental benefits*" to the practice of biocontrol, they knew (Memmott *et al.* 1994) but did not state in the paper the limitations and bias inherent in the work. One might fairly ask, would the paper have been cited >100 times if it had shown very low levels of parasitism? Would the results have been picked up and amplified in the popular press (including *Science* magazine "News of the Week"; *Science Daily*; and USA TODAY)? Compare it to the careful work published by Johnson *et al.* (2005) showing relatively minor impact of an introduced parasitoid in Hawaii, which was cited only 7 times.

The media have also amplified other published research addressing non-target impacts of biocontrol. For example, Ortega *et al.* (2004) reported that host-specific gall flies introduced for biocontrol of spotted knapweed were eaten by deer mice in Montana, leading to short term population increases in this known vector of hantavirus, a human pathogen (see also Pearson & Callaway 2003, 2006). This illustrates the fact that monophagy in a control agent, an attribute which forms the theoretical basis for much of biocontrol risk analysis (see Kuhlmann, this volume), is no guarantee against possible indirect non-target effects, such as food web subsidies.

However, does the press overplay the truth in this point? The New York Times called it "*the tip of the iceberg*," while Smithsonian Magazine reported that: "*the number of hantavirus-infected deer mice has skyrocketed*." National Wildlife Magazine said: "*Ultimately, it's a human choice: which is worse, the weed or the illness?*" [note: there has been no discernable increase in hanta virus in humans]. Nowhere is it adequately acknowledged that a population increase in mice (which even in Ortega *et al.* (2004) appeared to level out after 2 years) would most likely subside in the face of increased predation by snakes, owls, coyotes, weasels, etc.

Lady beetles introduced to the U.S. and Europe for control of aphids have also been in the news lately. In recent years several native species of coccinellids have become relatively rare, while a few introduced species have become widespread and

abundant, a correlation often laid at the feet of “biocontrol gone bad”. What has been mostly missing from the discussion, however, is the fact that the agro-ecosystems that were previously sustaining artificially large populations of some native coccinelids are themselves “unnatural” monocultures of exotic plant species (citrus, alfalfa) pumped up with fertilizers and irrigation, supporting outsized populations of other exotic (aphid) species, that happen to present a temporary lucrative resource exploited by native beetles. These exotic ecosystems are only a few hundred years old in North America; displacement of a native species from a transient resource in an alfalfa field should not be considered a negative environmental impact. Whether the deliberately introduced lady beetles displace native species in less disturbed natural habitats remains to be determined. As pointed out in a detailed analysis by (Harmon *et. al* 2007): “Even with multiple long-term data sets, it is currently difficult to make any general conclusions regarding the impact adventive coccinellids have had on native coccinellid assemblages.”

The fact is that non-target impacts of biological control have captured the imagination of the public, and of academics as well, in recent years. It has become a trend, leading to a wave of publications, grants, books, presentations, and press reports (Keith Warner, *in prep.*). There is some indication, however, that the wave of pique has peaked, and is now returning to a level more proportionate to actual risk levels. For example, the number of papers published in the journal *Biological Control* that contain the word “non-target” increased gradually over a decade and a half, but appears to have declined somewhat in the last few years:

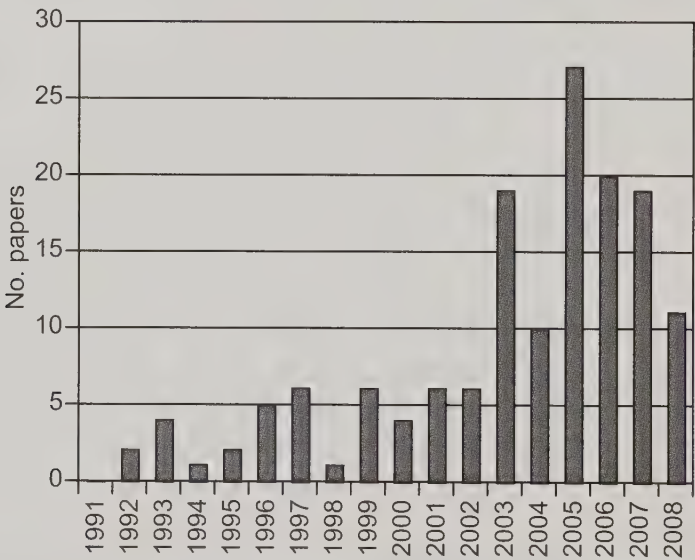


Fig. 2. Number of papers, by year, published in the journal *Biological Control* containing the word “non-target.”

The trend shown in this graph fits almost perfectly to the criteria used by Abrahamson *et al.* (1989) to characterize “Fads in Ecology.” They define these as “passing fashion, a craze, exaggerated zeal; a concept that receives more attention than it deserves”. The intense focus on non-target impacts of biological control has undoubtedly performed an invaluable service in countering the complacency of practitioners schooled during an era of different social values. However, the return to

a more balanced perspective may ease the burden on regulatory authorities to try to manage biological control risk to vanishingly small levels, especially in the face of so many other pressing environmental challenges.

CONCLUSIONS.

Classical biological control is operating in a new regulatory environment of stricter oversight in several countries around the world. There is not yet any evidence that this tighter supervision is leading to more effective or to safer implementation. In some cases, new regulations are creating bureaucratic obstacles to timely issuance of permits. The assessment of biocontrol risk is guided at least as much by social as by ecological considerations. The intense emphasis on non-target impacts appears to be a Fad in Ecology, which may be waning as a more balanced perspective comes to prevail.

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PHEROMONES, SEX ATTRACTANTS AND KAIROMONES IN WEED AND INSECT BIOLOGICAL CONTROL: AN EMERGING FRONTIER OF TOOLS TO MANAGE RISK AND REWARD

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ABSTRACT.

Pheromone traps can help to determine the establishment and population size of new pest and beneficial organisms, as well their phenology, synchrony with their host, dispersal, and rate of spread. The application of lures for biocontrol agents introduced against either insects or weeds is emerging as an expanding use pattern for semiochemicals. The kairomonal trapping of biocontrol agents including parasitoids and predators of scale insects represent the earliest examples of this new application for studying population trends. The deliberate development of a sex attractant for gorse pod moth (*Cydia succedana*) was probably the first example of the use of attractants in weed biocontrol, although there have now been several others developed and used after release of new organisms. After the lure was identified in Hawaii, trapping showed that the gorse (*Ulex europaeus*) biocontrol agent *Agonopterix umbellana* was established in New Zealand, and enabled field experiments to determine the minimum number of moth pairs required for establishment. The recent discovery of a newly-introduced parasitoid with attraction to the obscure mealybug pheromone showed that both can be monitored in one trap. Bioprospecting for new biocontrol agents using kairomones such as mealybug pheromones in the centre of biodiversity could help to offer a new risk mitigation tool for classical biocontrol. These cases of pheromones for monitoring biocontrol agents suggest that the area warrants development, especially since it also informs us about pest incursion biology. *Catoblastis cactorum* in Mexico has proven that some insects can be both successful biocontrol agents and unwanted pest organisms.

INTRODUCTION.

Inadequate post-release monitoring of biocontrol agents has long been recognized as a critical failing of biocontrol programs. Challenges to the ecological safety of biocontrol (Simberloff & Stiling 1996) have highlighted the need to document impacts on target and non-target organisms (Delfosse 1999). Unfortunately, monitoring insects released for biocontrol of weeds usually relies on sampling immature stages from plant material (e.g. Nagata & Markin 1986). This method is labor-intensive and insufficiently sensitive when insect populations are at low density.

By contrast, a method widely used and cost-effective for pest management in horticulture, agriculture, and forestry involves sampling adult insects using sticky traps baited with attractants such as pheromones. Pheromone traps or the kairomone (species-species) equivalent have been long and widely used to report

the establishment of many new pest organisms, with the largest application being gypsy moth (*Lymantria dispar*) (Linnaeus, 1759) in the USA, where hundreds of thousands of traps have been used over a long period (Sharov *et al.* 2002).

Attractants that work in the field, including moth sex attractants (which are essentially likely to be pheromones but where the female pheromone gland contents has not been confirmed) have been identified for a certain number of weed biocontrol agents or potential agents (e.g. Suckling *et al.* 1999). Furthermore, kairomones have long been known to be attractive for parasitoids (McLain *et al.* 1990; Morgan & Hale 1998) and predators of scale insects (e.g. Mendel *et al.* 1995; Dunkelbloom 1999), and there are at least 11 hymenopteran parasitoid pheromones known and many volatiles reported (El-Sayed 2008), representing potential for further examination. However, the exploitation of these findings through development into field programmes appears to be lacking momentum, with a few exceptions. Case studies will be used to illustrate a range of examples involving semiochemicals and biocontrol agents.

BENEFITS TO IPM.

There are various ways that attractants for biocontrol agents could be deployed within integrated pest management programs, but the additional complexity of the third trophic level makes the context very important to the outcome of pest suppression. It is possible to consider both monitoring tools and direct management of biocontrol agent populations, and there are important successes in both areas. As in the development and use of attractants for pests, usage in biocontrol may only result in increasing knowledge of ecosystems, rather than lead to direct uptake by growers and practitioners. Of course, there have been cases where odourant-based concepts have been tested without success at improving biocontrol. For example, attempts to attract a tachinid parasitoid into orchards with borneol did not result in reduced winter moth damage (Roland *et al.* 1995). Although there are so far relatively few examples of successful deployment of attractants in biocontrol, cases are emerging.

The identification of a kairomone inducing oviposition by the parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) (Millar & Hare 1993) was later used to increase parasitisation of California red scale in the field (Hare *et al.* 1997). Hare *et al.* (1997) suggested that exposure of *A. melinus* reared in commercial insectaries to O-caffeoyltyrosine prior to release may be a means to improve the effectiveness of *A. melinus* used in augmentative release programs to control California red scale.

In another well-developed case, the synthetic pheromone formulated from chemicals found in the airborne secretion of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae), the spined soldier bug (Aldrich *et al.* 1984), was found to be highly effective in attracting nymphs and adults of this beneficial predatory insect to desired areas for (bio)control of pests in the U.S.A. The dorsal abdominal glands are much smaller in adult females than males, and females produce a mixture of (*E*)-2-hexenal, (*E*)-2-octenal, (*E*)-2-hexenoic acid, benzaldehyde, and nonanal in these glands. The pheromone enables field collection for augmentative release (Thorpe and Aldrich 2005). There is similar work underway in this area in Japan (Mituzani 2005).

A relatively new area using the sampling efficiency of pheromone traps in IPM is their application in the area of biocontrol of horticultural pest insects. This was explored with the pheromone of a codling moth parasitoid *Ascogaster quadridentata* Wsml. (Delury *et al.* 1999), where phenology, synchrony, and presence of the pest and parasitoid were determined by pheromone trapping (Suckling *et al.* 2002). In this case, the nil tolerance of codling moth ultimately limited the scope of working with the parasitoid in New Zealand's export apple orchards. Parasitoid populations were not supported in these highly managed situations, where the host is kept rare. In practice, it would be necessary to offer growers specific benefits of monitoring their natural enemies before technology uptake could be expected. Instead, mating disruption of codling moth is increasingly being used to maintain levels of pest control. Hence the benefits of this approach may sometimes only be of new knowledge of phenology or regional geographic distribution.

Monitoring of a wider guild natural enemies has been examined using synthetic host induced plant volatiles, and ways of enhancing spring populations of natural enemies together with conservation biocontrol tactics have been proposed (James 2003). A wide range of predators and parasitoids (11 species) were attracted (James 2005).

Another example involving semiochemicals concerns self-introduced natural enemies gone wrong. The multi-colored Asian lady beetle, *Harmonia axyridis* (Pallas) became a considerable nuisance in the U.S.A. due to a habit of invading houses and other places in search of shelter to form overwintering aggregations. There is a search for repellents to prevent these unwanted invasions (Riddick *et al.* 2004). Similarly, the cactus moth *Catoblastis cactorum* (Berg), a biocontrol agent that provided spectacular control of *Opuntia* spp. cacti in Australia (Dodd 1940), has accidentally invaded the continental U.S.A., most likely from the Caribbean, and this moth now threatens indigenous cacti in the southern U.S.A. and Mexico (Hernandez *et al.* 2007). The pheromone has been identified as part of a management plan (Heath *et al.* 2006).

BENEFITS IN WEED BIOCONTROL.

There is obvious potential application for survey trapping in cases of weed biocontrol where Lepidoptera are involved. This order of insects is often used as weed biocontrol agents and there are many more attractants for Lepidoptera than other insects (El-Sayed 2008). The advantages of pheromone trapping in such biocontrol programs include low costs for materials and labor, ability to survey extensively in time and space, efficacy at low population density, high specificity for the target species, and high correlation with reproduction since adults are sampled (Suckling *et al.* 1999).

A low-cost method was tested to develop sex attractant traps for the gorse pod moth (*Cydia succedana*) and the gorse soft shoot moth (*Agonopterix umbellana*) (Suckling *et al.* 1999; 2000). This involved deductive screening of blends thought likely to be attractive, based on congeneric species (e.g. El-Sayed 2008), and tested as single components, binary, or ternary mixtures. In both cases, the method was successful. These new tools have been successfully used to support biocontrol of the gorse in New Zealand, representing the first time a sex attractant was used to document the successful establishment of a biocontrol agent within a country

(Suckling *et al.* 2000). This provides evidence that the biocontrol agent's life cycle was not optimally synchronized with the target weed with negative consequences for efficacy (Hill *et al.* 2000).

The identification of four sex attractants was attempted in Hawaii by deductive field screening of binary and ternary blends for potential use in the study of biocontrol agents against *Rubus* spp. and other weeds, but only two of the four experiments were successful. Attractants for *Croesia zimmermani* (Lepidoptera: Tortricidae) and *Schreckensteinia festaliella* (Lepidoptera: Heliodinidae) were thus further developed by deductive field screening of a variety of lures in sticky traps (Suckling *et al.* 2006). The subsequent use of the traps by Tracy Johnson (pers. comm.) has since included demonstration of the host range to include native Hawaiian *Rubus* spp. being attacked by the introduced agents at altitudes where only the native *Rubus argutus* is present (Gerrish *et al.* 1992), thus indicating range expansion geographically as well. The apparently beneficial risk-reward result of this low cost approach to providing a lure warrants further development for other Lepidoptera, but a study based on gland extracts is preferable. The approach has potential for other types of weed biocontrol agents (e.g. Cossé *et al.* 2006).

POST-RELEASE MONITORING.

Clear benefit can be identified from the development of monitoring systems based on sex attractants that can enable biocontrol programs to better evaluate the impacts of weed or insect control agents after release, which is widely requested by submitters to the Environmental Risk Management Authority of New Zealand (www.ermanz.govt.nz). The benefits were shown by researchers in a Landcare Research experiment where a mass release program investigated a range of *Cydia succedana* release densities (10 adults to 300 adults per site). Pheromone trapping established later that 5 pairs was sufficient, enabling many more sites to be targeted for release and establishment. An attractant also proved valuable for assessing the success of releases of *Agonopterix umbellana* around New Zealand, which depended on life stage (larvae or adults) released and size of release from 300-1000 adults and/or larvae (Gourlay pers. comm.). Recent examples of programs monitoring natural enemies with insect attractants are presented in Table 1.

Post-release monitoring of a new organism was serendipitously enabled when it was discovered that a mealybug parasitoid (*Pseudophycus maculipennis* Fachhandel), recently-released in New Zealand under the Hazardous Substances and New Organisms Act (Charles 2004) was attracted to obscure mealybug (*Pseudococcus viburni* Maskell) pheromone traps (Bell *et al.* 2008) that had been baited with synthetic pheromone (Millar and Midland 2007). In the North Island's Hawkes Bay, the phenology of the two species evident in the traps showed a mealybug generation without evident parasitoid catch in the middle of the winter. Traps recovered *P. maculipennis* from many sites in two regions where releases were been made, and its' rate of natural spread was determined from wider recoveries (Bell *et al.* in prep.).

Table 1. Benefits demonstrated of insect attractants in six case studies of biocontrol from New Zealand and Hawaii.

Case	Organism (family)	Benefits (lure type)	Reference
1	Gorse pod moth <i>Cydia succedana</i> (Tortricidae)	Presence/absence, synchrony with the host phenology (sex attractant), minimum release for establishment during national release programme (sex attractant)	Suckling <i>et al.</i> 1999 J. Memmott and H Gourlay pers. comm.
2	Gorse soft shoot moth <i>Agonoptrix ulcitella</i> (Oecophoridae)	Population presence in New Zealand for the first time, regional distribution, minimum release for establishment during national release programme (sex attractant)	Suckling <i>et al.</i> 2000, H. Gourlay pers. comm.
3	Codling moth egg parasitoid <i>Ascogaster quadridentata</i> (Braconidae)	Synchrony with the host phenology, national distribution, no IPM use (pheromone)	Suckling <i>et al.</i> 2002
4	Mealybug parasitoid <i>Pseudophycus maculipennis</i> (Encyrtidae)	Post-release survey spread distribution, (kairomone),	Bell <i>et al.</i> 2008
5	<i>Croesia zimmermani</i> (Tortricidae)	Distribution (sex attractant)	Suckling <i>et al.</i> 2006
6	<i>Schreckensteinia festaliella</i> (Heliodinidae)	Distribution (sex attractant)	Suckling <i>et al.</i> 2006

RISK REDUCTION IN CLASSICAL BIOCONTROL.

One under-exploited role for insect attractants in biocontrol that could be developed is based on the discovery of kairomonal attraction and the following hypothesis. If a cross species attraction has evolved between a natural enemy and its host/prey then the natural enemy may be more likely to be quite species-specific in host range. It seems unlikely that the trait of broadband reception of species-specific pheromone would occur at random, although it has to be noted that the phenomenon of attraction can occur in clusters within the same genera of predators (Mendel *et al.* 2004). On balance, the risk of a proposed new organism with the demonstrated evolutionary investment in detecting and responding to the pheromone of its exotic host species then tracking the habitat and/or the pheromone of an equivalent native species seems very low. Therefore the introduction of the new organism known to be tuned to the pheromone of its' intended target host should carry lower risk of non-target impacts. Species-specificity in kairomone communication is therefore likely to be a desirable trait for lowering risk in biocontrol.

This testable hypothesis leads to the idea of bioprospecting for new biocontrol agents using odourants, as a way of pre-screening candidates for biosafety.

The idea of bioprospecting for new biocontrol agents using kairomones is not new, since Mendel *et al.* (2004) suggested that it could lead to the discovery of new predators for pine blast scale which could be introduced into Israel. In their ecosystem, a whole guild of predators can be trapped. They found that two guilds of predators were attracted: flower bugs of the genus *Elatophilus* Reuter and brown lacewings of the genera *Hemerobius* and *Symphorobius*. Predators identified as attracted to the pheromone of the scale in Portugal were then evaluated to augment the natural enemy fauna of Israel.

We (DMS with S. Learmonth and J.G. Millar pers. comm.) have been exploring the potential of this concept in the centre of biodiversity of the long-tailed mealybug (*Pseudococcus longispinus*) in Western Australia. We have been using the newly-identified synthetic pheromone (J.G. Millar pers. comm.), although to date and possibly due to the long-term drought, no parasitoids have been trapped (Learmonth pers. comm.).

CONCLUSIONS.

The development and use of attractants for insect or weed biocontrol agents is emerging as an expanding use pattern for semiochemicals, and it is likely that new applications will be developed. Successful early cases of use of pheromones and other attractants for biocontrol agents suggest that the area warrants further consideration and development. Although weed biocontrol agents seldom have unpredicted non-target impacts, if they become unwanted organisms due to host range expansion for whatever reason, insect attractants can offer benefits in managing the reverse situation. *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) has emerged as a threat to native cactus biodiversity in the southern U.S.A. and Mexico, and illustrated that some insects can be both excellent biocontrol agents (Dodd 1940) and unwanted organisms. In this case, the pheromone was identified when it became an unwanted organism (Heath et al. 2006). In other cases with both pest and beneficial insects, pro-active identification of attractants could help to manage risks with new organisms that warrant this investment.

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BIOLOGICAL CONTROL OF PESTS AND WEEDS BY TARGETED DISRUPTION OF THEIR LIFE CYCLES

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ABSTRACT.

Perturbation analysis of matrix population models yields a prescription on how to intervene in an invading organism's life cycle to change rates of population growth and spatial spread. One application is controlling an invading organism by targeted disruption of its life cycle, which involves *prospective analysis* (using sensitivity and elasticity) to identify transitions in the life cycle that potentially influence population growth, and *retrospective analysis* (using contributions based on both variability and sensitivity of life cycle transitions) to identify which of these is most variable or amendable to manipulation. Here we compare applications of this technique in biological control of arthropods and weeds. We ask how robust is the prescription for intervention to changes in community structure (ranging from a single species pest population to a system with three interacting trophic levels)? We conclude that (1) sensitivity and elasticity structure can be used to improve the diagnosis of invader vulnerabilities, (2) the diagnosis is fairly robust to variation in community structure in at least one case, and (3) combining the manipulation of disturbance, colonization, local interactions (competition, mutualism, and natural enemies) can achieve biological control using fewer control-organism individuals and species, while helping to identify why we have invasions in the first place.

INTRODUCTION.

Invasive species threaten our environment and economy, and there is a growing body of knowledge for understanding, predicting, and managing the invasion process. Strategies for managing invasive species include predicting and preventing their entry into new areas, detecting them early and eradicating them, and mitigating them using various chemical, mechanical, and biological control measures. Biological control has a prominent role in mitigating invasive species problems, provided that we continue to strive for improvements in safety and effectiveness.

Observational studies of population dynamics repeated from time to time and place to place yield critical evidence of the pattern in invasions and the role of natural enemies in the population growth and spread of pests and weeds. They permit estimates of the degree of pest suppression caused by natural enemies and evidence of the persistence of pest-enemy interactions over the longer term. They may someday be complete enough to analyze time-series of population densities for evidence of natural enemy regulation of pest populations (Bjornstad & Grenfell 2001). However, passive observations alone generally won't do the trick. A system needs to be perturbed to reveal the forces that maintain it.

Experimental studies of population dynamics of pest-enemy interactions may be used to screen variables potentially influential on population growth rates of pests and weeds and establish the form of functional relationships. The driving forces of ecological systems are disturbance, colonization, and local organism interactions (predation, competition, and mutualism). If we can identify the qualitative and quantitative changes in these forces that give rise to invasions, it follows that we might control invaders by coordinated manipulation of these forces. Factorial experiments manipulating these forces permit estimation of their independent and interacting effects, and such experiments permit us to simultaneously investigate both causes and cures for invasions.

Mathematical modeling of invasions and biological control has helped build a theoretical foundation for assessing causes and cures of invasions. In modeling biological control, there is a need to go beyond the classical model forms, which are pitched at too strategic a level and fail to address the day to day questions faced by practitioners (Kareiva 1990). Since the time of earlier reviews of modeling in biological control (Barlow 1999; Gurr *et al.* 2000), matrix population models have been widely used to guide population management (whether the focus is harvesting, conserving, or controlling populations) (Caswell 2001). There is growing recognition of the potential value of specific (as opposed to general) models applied to particular systems to guide management decisions. Many modeling studies underscore this potential by making predictions or recommendations, but few if any have yet been shown to change how biological control is practiced. This may reflect absence of evidence, rather than evidence of absence, because biological control decisions are often not well-documented.

There needs to be a closer integration of observational, experimental, and modeling approaches. The more each relates to the other, the better each becomes. Here I outline a combination of observational, experimental, and modeling studies that can be used generally to study the causes and cures of invasive species problems and specifically to better identify and exploit pest and weed vulnerabilities in managing invasive species.

MATERIALS AND METHODS.

Matrix Population Models.

Computation of population growth and spatial spread rates and their sensitivity to changes in vital rates (e.g. growth, development, survival, reproduction, movement) is important for managing (harvesting, conserving, and controlling) populations. Matrix models are widely used for this purpose following well established methods (Caswell 2001), and most analyses to date exploit linear-deterministic matrix models to project long-term changes in population density assuming that environmental conditions and hence vital rates remain unchanged. Matrix models allow easy computation of the asymptotic population growth rate [the finite rate of increase λ , where the intrinsic rate of increase $r = \ln(\lambda)$] as the dominant eigenvalue of the projection matrix. λ succinctly summarizes the influence of vital rates on population performance. The dominant right and left eigenvectors of the matrix represent respectively, the stable stage distribution of the exponentially growing population and the reproductive value of individuals in different life-history

stages. The eigenvectors can moreover be used to compute the sensitivity of λ to changes in vital rates. Expressions for these measures of population performance (finite rate of increase and its sensitivity, stable stage distribution, and reproductive value) were first derived for constant environments and subsequently extended to environments in which vital rates vary periodically or stochastically or with population density.

Perturbation analysis of matrix population models yields a prescription on how to intervene in an invading organism's life cycle to change rates of population growth and spatial spread. The relevant application here is controlling an invading organism by targeted disruption of its life cycle, which involves *prospective analysis* (using sensitivity and elasticity) to identify transitions in the life cycle that potentially influence population growth, and *retrospective analysis* (using contributions based on both variability and sensitivity of life cycle transitions) to identify which of these is most variable or amendable to manipulation. Here we compare applications of this technique in some case studies of biological control of arthropods and weeds. We ask how robust is the prescription for intervention to changes in community structure (ranging in these studies from a single species pest population to a system with three interacting trophic levels)?

RESULTS AND DISCUSSION.

Biological Control of Ragwort.

The global hypothesis underlying this research is the Activation-Inhibition Hypothesis (McEvoy *et al.* 1993). It holds that persistent biological control results from a shift in the balance between the short-range activation of weed outbreaks by local disturbance and weed colonization, and their long-range inhibition by insect herbivory and plant competition. Observational studies across 42 sites and up to 12 years per site documented two key functions of biological control systems, the strong suppression of ragwort *Senecio jacobaea* L. (Asteraceae) populations after sequential introduction of the three natural enemy species [the cinnabar moth *Tyria jacobaeae* (L.) (Lepidoptera: Arctiidae), the ragwort seed-head fly *Botanophila seneciella* (Meade) (Diptera: Anthomyiidae), and the ragwort flea beetle *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae)] and the persistence of weed-enemy interactions at low average levels over longer temporal and spatial scales (McEvoy *et al.* 1991). Perturbation-response experiments conducted at a single site identified the forces that maintain ragwort populations at very low average levels and estimated the independent and interactive effects of soil disturbance, resource limitation, colonization, plant competition, and two natural enemy species (McEvoy & Rudd 1993; McEvoy *et al.* 1993). A Life Table Response Experiment (LTRE) combining the full factorial experiment and a stage-structured linear-deterministic matrix model (with 3 stages and 5 transitions) was parameterized for each of 24 treatment combinations using estimates from the field experiment (McEvoy & Coombs 1999). Bootstrap methods were used to estimate the uncertainty in vital rates due to sampling error, and confidence intervals were constructed for all measures of population performance (including finite rate of increases, sensitivities, and elasticities) (Dauer & McEvoy, unpub. data). The elasticity of λ was greatest to perturbations in the 'biennial transitions' of the ragwort life cycle except in some cases involving the cinnabar moth, where perturbations in the 'perennial transitions'

were more important. Feeding by cinnabar moth caterpillars induces perennality (having a life cycle lasting more than two years) and iteroparity (repeated production of offspring at intervals throughout the life cycle) in the ragwort life cycle, which can soften the impact of natural enemies; the ragwort flea beetle effectively blocks these life-cycle changes (McEvoy & Coombs 1999). We conclude that (1) sensitivity and elasticity structure can be used to improve the diagnosis of invader vulnerabilities, (2) the diagnosis is fairly robust to variation in community structure, and (3) combining the manipulation of disturbance, colonization, local interactions (competition, mutualism, and natural enemies) can achieve biological control using fewer control-organism individuals and species, while helping to identify why we have invasions in the first place.

Biological Control of Garlic Mustard.

A linear-deterministic model of garlic mustard *Alliaria petiolata* (M. Bieb.) Cavara & Grande (Brassicaceae) (featuring 3 life stages and 5 life-cycle transitions) was developed and analyzed to guide the selection of biological control organisms from a pool of four candidates (Davis *et al.* 2006). The candidates consist of four related weevil species (Coleoptera: Cucurionidae) that attack rosettes (*Ceutorhynchus scrobicollis* Neresheimer & Wagner), stems (*C. roheri* Gyllenhal, *C. alliariae* Brisout), and seeds (*C. constrictus* (Marsham)) of *A. petiolata*. The rank order of elasticities associated with transitions was robust to simulated variation in model parameters. The authors concluded that population growth is most sensitive to rosette mortality and reduction of seed output and that combinations of agents will be required to reduce these transitions and suppress rapidly growing garlic mustard populations.

Biological Control of Codling Moth.

An analysis of a linear-deterministic matrix model of the codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae Olethreutinae) (Mills 2005) identified survival of the second instar and cocoon stages of the pest life-cycle as potentially most influential on population growth and most easily manipulated using parasitoids (based on prior knowledge of the life stages attacked by each parasitoid species). The projection matrix was comparatively large, involving 18 transitions among 9 stages in the codling moth life cycle, and parameters were estimated from literature sources spanning multiple populations. The elasticities reported were the subset potentially influenced by parasitoids; whether the rank order of elasticities is sensitive to uncertainty in the vital rates was not examined. In addition to identifying and exploiting pest vulnerabilities, this study counseled avoiding antagonistic interactions among natural enemies; spatial, temporal, and behavioral refuges that might protect the pest from parasitism; and parasitoids with overlapping niches.

Comparing Native and Invasive Plant Species.

The growing number of studies using matrix models of invasive species is helping to create a more reliable basis for comparison, interpolation, and extrapolation across case studies. Using an inductive approach, it is now possible to ask: Do elasticity and sensitivity patterns vary with environment? Do native and introduced species and populations have different patterns of sensitivity and

elasticity? Without general expectations and guidelines we are left to parameterize matrix models for each invading population on a case-by-case basis. If elasticity patterns differ for native and invasive species and populations, then it becomes more difficult to predict how invaders might behave in their adopted home from studies conducted in their native home.

A recent review compared population dynamics between invasive and native species using published linear-deterministic matrix population models for 21 invasive and 179 native plant species (Ramula *et al.* 2008). The goals were to examine whether the population growth rate responsiveness to survival, growth and fecundity perturbations varied between invasive and native species, and to determine which demographic processes of invaders to target for reductions in population growth rate. Invaders had higher population growth rates (λ) than natives, resulting in differences in demographic processes. Elasticities of λ to growth and fecundity transitions were higher for invaders; elasticities to survival were greater for natives. For rapidly growing populations of short-lived invaders, growth and fecundity transitions should be prioritized as control targets over survival transitions. For long-lived invaders, simultaneous reductions in more than one demographic process (preferably survival and growth) are usually required to ensure population decline. These general guidelines can be applied to rapidly growing new plant invasions and at the invasion front where detailed demographic data on invasive species are lacking. They can also be applied to the questions whether biological control can curb an upsurge in pest abundance and prevent a wider epidemic (McEvoy & Coombs 1999), or whether biological control can slow or reverse the spread of an invading population (Fagan *et al.* 2002). With more case studies, it may be possible to supplement this comparison of native and invasive *species* with contrasts of native and invasive *populations* of the same species.

CONCLUSIONS.

It is possible and profitable to simultaneously address the causes and cures of biological invasions. We recommend a combination of observational, experimental, and modeling approaches. The more each relates to the other, the better they individually and collectively become. This approach can inform management of invasive species whether by prediction and prevention, early detection and eradication, or mitigation of established invaders (chemical, mechanical, and biological control).

Perturbation approaches applied in experiments and models offers new, robust ways of identifying and exploiting pest/weed vulnerabilities. The practical aim is to create a biological control technology that is economic, effective, persistent, and safe. The theoretical aim is to create a general theory of biological control (covering predators, pathogens, parasitoids, and herbivores as natural enemies) that makes investigating individual cases easier.

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ECOLOGICAL THEORY VS. PRACTICE: HAVE NON-TARGET CONCERNS LED TO INCREASED USE OF MONOPHAGOUS AGENTS?

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ABSTRACT.

The last 25 years have seen an important conceptual shift in arthropod biological control with respect to awareness of potential negative effects. Risk assessment for non-target impact has become one of the key elements of agent selection since it is now almost universally recommended that released agents should demonstrate high host/prey fidelity. Nonetheless, non-specific agents continue to be released in modern biological control initiatives. Our first objective was to review several selected examples of classical arthropod biological control introductions to assess the frequency with which monophagous species have been introduced for pest suppression in the last decade. The second objective was to explore the types of selection decisions faced by researchers to elucidate the various motives behind using non-specific agents. These decisions are influenced by multiple interacting factors such as the number of candidate agent species and a researcher's interpretation of acceptable non-target risk.

INTRODUCTION.

Classical biological control is an important, cost-effective pest management strategy (Bale *et al.* 2008) in the face of incessantly emerging pest problems associated with exotic species and the increasing restrictions on conventional pesticide use. However, despite its long history of use and century-old reputation as a "green" approach, the theory and practice of arthropod biological control has become enshrouded in controversy following the revelation that non-target effects may be more common than initially perceived (Howarth 1991; Lynch *et al.* 2001; Kimberling, 2004). Intense global discussions during the last two decades have clarified the importance of using host-specific agents to minimise environmental risk (Onstad & McManus 1996; Louda *et al.* 2003), particularly whenever permanent establishment of the agent is desired (i.e., classical biological control). For the rest of the discussion on biological control, we will be referring solely to the release of parasitoids within the context of classical biological control.

The Code of Conduct for the Import and Release of Exotic Biological Control Agents (International Standards for Phytosanitary Measures No. 3) is said to have changed the way many countries evaluate potential agents for introduction (Kairo *et al.* 2003). But what effect has it actually had on the specificity of agents released most recently? If it is true, as suggested, that the heavy scrutiny of biological control has led to more careful selection of agents (Henneman & Memmott 2001), this increase in specificity should by now start to be apparent if we compare the species being approved in classical biological control programmes today to those that were

released prior to the conceptual shift. The first objective of this paper was to review a subset of the classical biological control introductions conducted over the last decade and determine what proportion of approved agents is strictly monophagous. Similar reviews have been conducted in the last decade, however, they differ in fundamental ways. The extensive lists of American and worldwide agent introductions assembled by Kimberling (2004) and Stiling (2004), respectively, only cover programmes prior to 1981. The review executed by Frank & McCoy (2007) is significantly more updated, including releases up to and including 2003, however it deals solely with programmes in the state of Florida. It is therefore a good opportunity to assess how biological control practitioners around the world are adapting to the new “climate” of agent introductions. Our second objective was to illuminate the various motives of practitioners who opt for less specialised agents.

MATERIALS AND METHODS.

Clarification of Host Specificity Vocabulary.

Before commencing with an analysis of the specificity of agents released, it is necessary to touch on the subject of nomenclature. Consistency of terminology is essential for precise expression, which in turn prevents confusion when interpreting data. Not for the first time in the field of biological control (see Eilenberg *et al.* 2001; Frank & McCoy 1990), certain key words have lost the rigidity of their meaning. Among entomologists, expressions like “monophagous”, “oligophagous” and “polyphagous” have been employed as a simplified quantification of insect host ranges. These terms have been used for decades by biological control practitioners, however their use has been evolving in different directions and this divergence is readily apparent in recent publications. In reality, the only species that can easily and unanimously be assigned to such a category are the truly specific ones attacking a single host species (monophagy in the strictest sense). Agents utilising more than one host are said to express some degree of polyphagy and it is within this vast group that inconsistencies in word usage have arisen.

Tolerance of “loose” host range vocabulary is detrimental because it impedes our ability to assess the importance of host specificity in biological control. For instance, if two hypothetical authors had personal interpretations of “oligophagous”, it would be impossible to unite their independent studies: one that calculates that 40% of agents released are oligophagous and a second study showing that oligophagous agents have a 60% percent chance of non-target effects. Among recent studies that have analysed the use of specialist and generalist agents, there is unfortunately very little consistency in the use of terminology and therefore in the division of the specificity continuum (compare Kimberling 2004; Stiling 2004; Frank & McCoy 2007). Since one of our primary objectives is to determine what minimum degree of specificity is sufficiently safe for biological control, it is necessary to have consensus on how we define degrees of specificity.

With the possible exceptions of monophagous and specialist, words like oligophagous, polyphagous and generalist were not originally intended to equate to specific grades of specificity. Besides which, there are far more potential classes of host specificity than there are such terms, unless we begin to invent new expressions to indicate intermediate degrees of specificity (e.g., “hemi-polyphagous” *sensu* Frank

& McCoy 2007). We recommend that the use of these vague expressions be restricted to cases where it is safe to generalise about specificity. However, all analyses that require placing agents into discrete groups should use more self-explanatory terms: “species-specific”, “genus-specific”, “family-specific”, etc. This suggested vocabulary has already been used by some arthropod biological control scientists (e.g. Kenis *et al.* 2005), but has not been broadly adopted. It is superior because, although it uses the same principles as previous systems, it is self-explanatory by explicitly stating the lowest taxonomic group that includes all species within an agent’s host range. It of course shares the same flaw as other schemes in that it excludes information about the number of host species; however, until proven otherwise, we will continue to put emphasis on phylogenetic diversity of hosts rather than number of hosts attacked. We use this system in the following discussion of the specificity of agents released in recent years.

Specificity of Modern Agents.

We conducted a simple review of recent classical biological control releases to determine whether practitioners are continuing to use entomophagous agents with generalist tendencies despite evidence that these pose the greatest risk to novel environments (Kimberling 2004). It was not our goal to produce as exhaustive a review as in the examples listed above. Instead, we narrowed our search using specific criteria to obtain a sample of 20 agent introductions orchestrated by different researchers from multiple countries and continents. The criteria for our search were as follows: (1) we considered only target pests that attack “fruit”-bearing trees/vines (included are pests of apple, citrus, coconut, coffee, grape, mango, olive and peach); (2) agents were included only if released into a novel region in 1996 or later (our examples range from 1996 to 2006); and (3) in cases where multiple agents had been released against a pest as part of a single programme, only the most recently released agent was used. This was to prevent a bias arising from over-representation of certain researchers or programmes. An exception was made for the pest *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) since several practitioners from different countries made similar decisions to release the same group of agents.

In many cases, agents released since 1996 had been previously released in other countries. We assumed that all agents released in the last decade underwent some degree of evaluation by the receiving country; therefore, we feel justified to treat these cases as novel introductions. For most of the agents reviewed, the year and location provided was based on the most recent known introduction. However, there were five instances where an agent had been released by several countries over a relatively short period and for these introductions we simply used the date of the most recent introduction and indicated “multiple” locations. Since a large proportion of the most recent and well-documented releases against fruit pests have taken place in the United States, this country is somewhat over-represented in this type of survey. Nonetheless, these classical biological control projects are often operated by a large number of scientists, meaning that no one individual would have significantly affected the results of this survey.

We calculated a host specificity rank (see Table 1 footnote for explanation) for each agent included in our review using host range data available in the literature and on government and research websites. For a few of the agents, there was very

limited host range data available, meaning that estimations of specificity are likely not perfectly accurate. For this reason, we divided the specificity continuum into five basic classes based on whether an agent's hosts all belonged to a common species, genus, family, order or class. This review lacked the information necessary to create an even finer scale using tribe, subfamily, suborder, etc.

Motives for Using Non-specific Agents.

After clarifying that the majority of recently introduced agents are not species-specific, it was necessary to elucidate the rationale behind decisions to use higher-risk agents. Unfortunately, this kind of information is not accessible for most of the agents that we reviewed. We therefore listed several *possible* reasons for using non-specific agents and briefly discussed the validity of those arguments.

CABI Case Studies.

We also took a close look at the types of filters used in the early process of paring down the list of candidate agents following exploratory surveys. Since this information is not readily available for most programmes, we reported on our own experiences with four recent biological control programmes with which CABI Europe-Switzerland was involved.

RESULTS AND DISCUSSION.

Specificity of Modern Agents.

We identified approximately 50 entomophagous agents that had been introduced into new regions around the world since the mid-1990's to combat arthropod pests of "fruit"-bearing trees and vines. This list was thinned, as explained above, by removing all but the most recently released agents from specific programmes. We also omitted agents for which there were no sound data to determine degree of specificity. The 20 agents contained in the final list (Table 1) belong to 8 different families but are, coincidentally, all Hymenoptera and dominated by the Chalcidoidea. Of the 13 pest species listed, nine belong to Hemiptera, highlighting the importance of this group in the fruit industry.

Only a small proportion of classical biological control agents released in the last decade are suspected or proven to be species-specific (20% of cases shown in Table 1). In fact, we found that agents of all degrees of specificity continue to be redistributed around the world. The dominance of American examples in this review did not appear to skew the average specificity in either direction since agents of all ranks were introduced into the United States. One might have expected to see a trend of increasing agent specificity over this 10-year period since the importance of pre-release risk assessments has been gradually building during this time. When the specificity ranks in Table 1 were plotted against the corresponding year, no relationship emerged ($P = 0.201$). One observation made, however, was that all agents with the highest polyphagy (values 4 and 5) were released prior to 2000, while all subsequently introduced agents were at least family-specific. Whether this

Table 1. List of classical biological control agents included in our review. All agents shown were released in novel regions between 1996 and 2006.

Agent	Pest	Rank	Year Location
<i>Ageniaspis citricola</i> Logvinovskaya (Hymenoptera: Encyrtidae)	<i>Phyllocnistis citrella</i> Stainton (Lepidoptera: Gracillariidae)	1	1996 multiple
<i>Allotropa</i> sp. nr. <i>mecrida</i> (Walker) (Hymenoptera: Platygasteridae)	<i>Maconellicoccus hirsutus</i> (Green) (Hemiptera: Pseudococcidae)	1	2003 California
<i>Tamarixia radiata</i> (Waterston) (Hymenoptera: Eulophidae)	<i>Diaphorina citri</i> Kuwayama (Hemiptera: Psyllidae)	1	2000 Florida
<i>Anagrus epos</i> Girault (Hymenoptera: Mymaridae)	<i>Homalodisca vitripennis</i> (Germar) (Hemiptera: Cicadellidae)	2	2006 California
<i>Aphelinus mali</i> (Haldeman) (Hymenoptera: Aphelinidae)	<i>Eriosoma lanigerum</i> (Hausmann) (Hemiptera: Aphididae)	2	1997 Afghanistan
<i>Encarsiella</i> sp. D (Hymenoptera: Aphelinidae)	<i>Aleurodicus pulvinatus</i> (Maskell) (Hemiptera: Aleyrodidae)	2	1998 Nevis
<i>Gonatocerus fasciatus</i> Girault (Hymenoptera: Mymaridae)	<i>Homalodisca vitripennis</i> (Germar) (Hemiptera: Cicadellidae)	2	2002 California
<i>Anagyrus kamali</i> Moursi (Hymenoptera: Encyrtidae)	<i>Maconellicoccus hirsutus</i> (Green) (Hemiptera: Pseudococcidae)	3	2002 multiple
<i>Gonatocerus ashmeadi</i> Girault (Hymenoptera: Mymaridae)	<i>Homalodisca vitripennis</i> (Germar) (Hemiptera: Cicadellidae)	3	2005 Tahiti
<i>Gyranusoidea indica</i> Shaf., Al. & Ag. (Hymenoptera: Encyrtidae)	<i>Maconellicoccus hirsutus</i> (Green) (Hemiptera: Pseudococcidae)	3	2002 multiple
<i>Lipolexis oregmae</i> Gahan (Hymenoptera: Aphidiidae)	<i>Toxoptera citricida</i> Kirkaldy (Hemiptera: Aphididae)	3	2000 Florida
<i>Neodryinus typhlocybae</i> (Ashmead) (Hymenoptera: Dryinidae)	<i>Metcalfa pruinosa</i> (Say) (Hemiptera: Flatidae)	3	2000 multiple
<i>Pauesia antennata</i> (Mukerji) (Hymenoptera: Braconidae)	<i>Pterochloroides persicae</i> (Cholod.) (Hemiptera: Aphididae)	3	1997 Yemen
<i>Phymastichus coffea</i> LaSalle (Hymenoptera: Eulophidae)	<i>Hypothenemus hampei</i> (Ferrari) (Coleoptera: Scolytidae)	3	1997 Columbia
<i>Psytalia concolor</i> (Szépligeti) (Hymenoptera: Braconidae)	<i>Bactrocera oleae</i> (Rossi) (Diptera: Tephritidae)	3	2003 California
<i>Cales noacki</i> (Howard) (Hymenoptera: Aphelinidae)	<i>Aleurothrixus floccosus</i> (Maskell) (Hemiptera: Aleyrodidae)	4	1996 Uganda
<i>Aprostocetus gala</i> (Walker) (Hymenoptera: Aphelinidae)	<i>Diaprepes abbreviatus</i> (L.) (Coleoptera: Curculionidae)	5	1999 Florida
<i>Cirrospilus ingenuus</i> Gahan (Hymenoptera: Eulophidae)	<i>Phyllocnistis citrella</i> Stainton (Lepidoptera: Gracillariidae)	5	1998 multiple
<i>Citrostichus phyllocnistoides</i> (Naray.) (Hymenoptera: Eulophidae)	<i>Phyllocnistis citrella</i> Stainton (Lepidoptera: Gracillariidae)	5	1999 multiple
<i>Semiolacher petiolatus</i> (Girault) (Hymenoptera: Eulophidae)	<i>Phyllocnistis citrella</i> Stainton (Lepidoptera: Gracillariidae)	5	1997 multiple
Rank (specificity): 1 = species-specific 3 = family-specific 5 = class-specific; 2 = genus-specific 4 = order-specific			

is true of all parasitoids released since the turn of the century is difficult to say given our small sample size and restricted pest category. Nevertheless, that is precisely the kind of change we should expect to see first (i.e., automatic rejection of the most polyphagous agents), whereas there will naturally be greater reluctance to ignore agents with intermediate specificity. Frank & McCoy (2007) similarly described a trend of reduced releases of generalist predatory beetles in Florida, although their analysis included a much larger number of species and longer time span.

In his review of parasitoids and predators introduced around the world prior to 1978, Stiling (2004) calculated that 22.5% of the agents were species-specific. This number corresponds moderately well with our estimated value of 15%. Stiling also noted that “generalists” (agents attacking multiple genera) comprised 48% of all releases. The current study shows that a startling 65% of recently used agents attack more than one genus. While the margin of error in our estimate is too great to be certain that generalist agents are used more now than before, these data clearly illustrate that ecological theory has not had a strong influence on this aspect of biological control practice.

Motives for Using Non-specific Agents.

Why do classical biological control practitioners continue to use non-specialised agents despite mounting evidence of the ecological risks? The answer to this question depends on the person providing it since there is no universally adopted benchmark for acceptable risk. Below, we list several possible reasons why monophagy is not a ubiquitous trait of released agents and discuss the likelihood that these arguments are used today.

1. No concern for non-target effects. It seems hardly possible, given our knowledge of ecosystem services and the value of biodiversity, for such a sentiment to persist. In our opinion, this attitude is extinct among professional biological control practitioners.

2. Insufficient or no host range data obtained. Although agent specificity can be roughly estimated using previous records in the literature (Sands & Van Driesche 2004), it is often necessary to test an agent with native non-target species from the area of introduction. Some form of official pre-release risk assessment was conducted by at least 30 of the 42 countries surveyed by Kairo *et al.* (2003). It is further possible that practitioners consider the risks of release even when they are not obliged to do so. However, it is difficult to know what level of host range testing is sufficient, since we can only test a representative sample of all potential non-targets. With evidence that parasitoids are more likely than herbivores to have disjunct host ranges (Messing 2001), specificity tests incorporating only a small number of non-targets risk overestimating specificity.

3. Belief that polyphagous agents are superior. There has been a great deal of research on natural enemy traits that promote successful control of pests, with a large focus on monophagous vs. polyphagous agents (e.g., Hassell & May 1973). Even though monophagous species may be more successful once established (Kimberling 2004), many classical biological control agents fail to establish and persist in novel environments (Stiling 1990). Thus, despite concerns of non-target

effects, some practitioners show a lingering interest in agents that demonstrate generalist tendencies (e.g., Lim *et al.* 2006) as this is believed to increase the probability of establishment.

4. No monophagous species available. Zwölfer (1971) suggested that monophagous natural enemies may be rare in nature. Given the narrow window of time and often limited geographic range of foreign exploration for agents, it is not surprising if field surveys frequently fail to identify monophagous candidates, whether they exist or not.

5. Monophagous agents too difficult to rear/mass-produce. The frequency of insect rearing papers in the literature is a testament to the difficulty of maintaining cultures. Not only are certain species difficult to sustain for long periods under artificial conditions, but several have proved impossible to even establish a colony. Rearing failures can arise from insufficient starting numbers, failure to stimulate mating, failure to provide the right food, etc.

6. Agent monophagous in area of introduction. If describing an agent's host range without reference to a particular region, we generally assume this to mean the host range over the agent's global distribution. However, if the availability of potential hosts varies between regions, then the region-specific host ranges of the agent should also vary and be equal to or smaller than its global host range. Thus, an agent known to be polyphagous in its area of origin can be functionally monophagous if its novel environment contains only the target host. This line of reasoning has often been used to justify agent introductions (e.g., Grandgirard *et al.* 2007). It is perhaps too early to know whether there is a risk in allowing the receiving environment to make an agent functionally monophagous. There are at least two possible negative outcomes: (1) due to poor inventories of insect fauna in most countries, it is possible that not-yet-described species would serve as hosts for non-specific agents and (2) introducing non-specific entomophagous agents could diminish the potential for future introductions of weed biological control agents.

7. Non-targets attacked are all adventive. In their assessment of the specificity of agents released in Florida, Frank & McCoy (2007) distinguished between agents that attack native and adventive hosts. While there may be little concern about increased mortality of non-native species, particularly invasive ones, the more ways in which an agent interacts with its environment, the more likely it is to have an impact, such as direct or indirect competition with native natural enemies.

8. Risk-benefit analysis gives green light. Most cases of introductions of generalist agents in the last decades have likely involved some manner of risk-benefit comparison. In most cases, such an analysis is informal and qualitative and perhaps never even documented. This is largely because too little is known about the receiving environment to predict potential negative consequences. Furthermore, although crop yield loss is easily quantified, it is exceedingly difficult to apply a monetary value to environmental impacts (Bigler & Kölliker-Ott 2006). There are recent examples where pests were so detrimental to the economy that predictable non-target effects were deemed acceptable (see examples in Cock 2003). When compared to the options of doing nothing or using chemical controls, it may be justifiable to use a non-specific agent.

CABI Case Studies.

There is a tendency to assume that the selection of an agent for release is based on rigorous efficacy and specificity trials, however, the fate of most potential agents is actually determined shortly after surveying and often based on scant data. Therefore, it can be argued that the proposal to introduce a generalist agent is largely the product of decisions made in the early stages of a programme, assuming of course that a number of natural enemies were initially identified. A simple literature search will uncover dozens of articles depicting foreign exploration for natural enemies and studies on the rearing, evaluation and release of “promising” agents. However, almost always absent is any publicly available discussion on the motives behind early decisions to select certain natural enemies for further evaluation while abandoning the rest. A close look at programmes conducted by CABI Europe-Switzerland on behalf of Canada provides some insight into the criteria used to isolate what are considered to be the most promising candidates.

A project was created in the 1990's to find a classical biological control agent for the European apple sawfly, *Hoplocampa testudinea* (Klug) (Hymenoptera: Tenthredinidae). Canada had expressed interest in the European parasitoid *Lathrolestes ensator* (Brauns) (Hymenoptera: Ichneumonidae) at the start of the programme, based on information in the literature. However, when field collections in Europe revealed three parasitoid species attacking the pest, scientists at CABI decided to conduct a study to determine which of two (the third species was never established in a culture) would be most suitable for classical biological control. Ultimately, *L. ensator* proved to be the more specific, well-synchronised and effective candidate and was released in Canada.

Beginning in the late 1990's, surveys were conducted in central and northern Europe to obtain potential agents for the cherry bark tortrix, *Enarmonia formosana* (Scopoli) (Lepidoptera: Tortricidae). At least 13 parasitoid species were obtained during three summers of surveys. Of these, *Campoplex dubitator* Horstmann (Hymenoptera: Ichneumonidae) was by far the dominant parasitoid, comprising 86% of all specimens collected (Jenner *et al.* 2004). All the remaining species were collected in such low numbers that it was not possible to establish colonies with them. Thus, all subsequent research has focused on *C. dubitator*.

Renewed interest in classical biological control of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), in the late 1990's led to a major research project on candidate European agents. As quite a lot was already known of this pest's parasitoids, the decision was made from the outset to focus on two of the three most dominant species (the third having already been introduced into Canada). The parasitoids *Trichomalus perfectus* (Walker) and *Mesopolobus morys* (Walker) (Hymenoptera: Pteromalidae) were known to cause 80-90% parasitism in beetle larvae (Williams 2003) but little was known of their host specificity. The aim was to measure the host ranges of both species, hoping that at least one would be sufficiently specific for release in Canada.

Exploration for parasitoids of leek moth, *Acrolepiopsis assectella* (Zeller) (Lepidoptera: Acrolepiidae), commenced in 2004 in Switzerland. Three years of field

sampling yielded six parasitoids. Identification of the parasitoids revealed that five were reported in the literature to be highly polyphagous while one, *Diadromus pulchellus* Wesmael (Hymenoptera: Ichneumonidae), was reputedly species-specific. Although three of the species were collected in sufficient numbers to establish cultures, only that of *D. pulchellus* was maintained for study. The decision to focus research efforts on this agent was made easier by the fact that it was the most abundant and consistent parasitoid on leek moth and a congeneric species, *D. collaris* (Gravenhorst), has already been used in many classical biological control programmes with proven efficacy (Sarfraz *et al.* 2005).

CONCLUSIONS.

Our literature review and assessment of agent selection criteria demonstrate that parasitoids used in classical biological control are primarily not species-specific. This is despite the fact that researchers are generally aware of non-target effects and give priority to specific candidate agents. One likely cause is that the specific agents that we should theoretically be using simply might not exist or might occur in such low numbers that establishing a high-quality culture is not possible. Hence, it is not surprising that researchers pursue non-specific agents. Longer survey trips to more regions of a pest's native range should increase the pool of natural enemies from which to select candidate agents, which could, in turn, increase the average specificity of agents released. However, we will likely first need to see evidence of this payoff before programmes begin devoting more resources to exploration.

Given the reasons stated by most practitioners to release non-specific agents and their shared interest in maintaining the green image of classical biological control, we conclude that most modern releases of non-specific agents are based on carefully made decisions. We also predict that the trend of using non-specific agents will continue along roughly the same trajectory, unless regulatory processes drastically restrict the allowable level of polyphagy. Currently, most regulatory agencies seem to realise the complexity of classical biological control programmes and that the definition of acceptable risk should be case-specific, depending largely on the severity of a pest problem and the risks of alternative control measures.

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SESSION 2

BIOLOGICAL CONTROL AND ENVIRONMENTAL/CLIMATE CHANGE

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ABSTRACT.

Arthropods have limited ability to regulate their body temperature and are thus subject to regional and seasonal changes in climate across their distributions. Insects and mites show wide variations in their ability to tolerate low temperatures and in their threshold temperatures for development, reproduction and activity. The interaction between these physiological attributes and climate explains the observed distributions and abundance of species and their relative success. In the context of biological control, temperature has a dominant effect on establishment – whether this is a pre-requisite for success as in classical control, or potentially undesirable as with glasshouse releases - and can have a profound influence on host-parasitoid and predator-prey relationships. The need to develop a greater understanding of the thermal biology of pests and control agents comes at the same time as climate warming is having demonstrable impacts on the range margins of species across both trophic levels, and the areas of cultivation of different crops. Importantly, our ability to predict the likely effects of global climate change requires a sound knowledge of pest-natural enemy-climate interactions under current regimes, and for most species of interest, this information is lacking. This session addresses this issue in papers that collectively highlight recent developments in methods that are used to study the various effects of temperature on biocontrol agents (including novel organisms), and on species where climate has a demonstrable effect on their distribution and success.

THERMAL ECOLOGY OF INVERTEBRATE BIOLOGICAL CONTROL AGENTS: ESTABLISHMENT AND ACTIVITY

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ABSTRACT.

Terrestrial arthropods have limited ability to regulate their body temperature. For this reason, many aspects of the biology and ecology of insects and mites, both pest species and their natural enemies, are influenced by temperature, ranging from macrophysiological effects on their distribution to more localized 'rate-based' responses, affecting processes such as development, reproduction, activity and survival. In the context of biological control, temperature is a major determinant of establishment, which is essential for classical agents but considered undesirable for species released for inundative control in glasshouses, and also has a direct effect on the activity of predators and parasitoids relative to their target prey. This paper describes advances in methods for assessing the establishment potential and activity of biocontrol agents, a focus of considerable interest in an era of global climate change.

INTRODUCTION.

Insects and mites are poikilothermic animals with limited ability to regulate their body temperature. For this reason, temperature can exert a major influence on such arthropods: at a macrophysiological scale, determining distributions and abundance, and at a more localized level, affecting 'rate-based' processes such as development, reproduction, activity and survival. Interest in the thermal biology of insects has increased greatly in recent years, stimulated largely by concerns about the possible impacts of climate warming. There are now well documented reports of shifts in the distribution of indicator species such as butterflies and other taxa of conservation importance (Parmesan *et al.* 1999; Thomas *et al.* 2001), as well as the range expansion of many pest species such as the pine beetle *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae) in North America (Tran *et al.* 2007), the southern green stinkbug *Nezara viridula* L. (Hemiptera: Pentatomidae) in Japan (Musolin 2007), the pine processionary moth *Thaumetopoea pityocampa* (Dennis and Schiff.) (Lepidoptera: Notodontidae) in the Italian Alps (Battisti *et al.* 2005) and the birch-defoliating moths *Epirrita autumnata* L. (Lepidoptera: Geometridae) and *Operophtera brumata* Bkh. (Lepidoptera: Geometridae) in northern Scandinavia (Jepsen *et al.* 2008). Whilst higher temperatures might be expected to increase rates of development and fecundity and reduce the winter mortality of pest species, similar effects would also impact on natural enemy species, thus the 'net outcome' of such interactions in an era of climate change is as yet largely unknown. However, a cautionary conclusion would be that climate warming would allow at least some pest insects and mites to increase in abundance and to expand their range margins beyond current distributions. Such changes are also occurring against the backdrop of the progressive withdrawal of pesticides without replacement, concerns about the environmental impact of GM crops, and relatively high development and registration

costs for biopesticides. The opportunity to utilize arthropod biocontrol agents is therefore as promising and opportune as at any time in the history of this technology.

Whilst the socio-economic importance of crop losses to pests and the possible exacerbating effects of a changing climate highlight the need to seek 'accurate predictions' on the future distributions and abundance of key species, the reliability of such forecasts is compromised by a number of factors. Firstly, it is difficult to predict with any accuracy the impact of future climate scenarios without a detailed knowledge of a species' ecophysiology and thermal ecology under prevailing climates, and for many species, this information is still lacking. As an example, the environmental risk assessment of non-native biocontrol agents intended for release in the UK in the 1990s rarely contained any direct measure of cold tolerance or winter survival; rather, 'climate matching' was used as a proxy for cold hardiness and it was assumed that species of tropical origin would not survive through temperate winters – an assumption now known to be untrue. Secondly, long term datasets (30-50 years) that represent a 'biological record' of responses to a changing climate are relatively rare (see earlier examples), especially so for natural enemy species. Also, direct experimental manipulation of climate (solar domes, FACE systems) and modeling approaches have rarely considered the 'biological control' dimension, and in any case, suggest that species-specific differences in life cycles, host plants, feeding guild and natural enemy interactions make it difficult to reach any general consensus.

The effects of current and future temperature regimes on pests and their predatory and parasitoid fauna are therefore many and varied, but a case can be made that two of these processes are of fundamental importance to the success and wider environmental effects of biocontrol: establishment and activity. Establishment is a 'double-edged sword' in biological control – for classical control, establishment is essential, but for inundative control in glasshouses, the likelihood of escape and establishment in the natural environment is undesirable (because negative impacts on non-target native species may occur), and in some countries would prevent the granting of a licence to release. With regard to activity, all insects, mites and their natural enemies have temperature thresholds below which the organism cannot move (walk or fly); above this threshold, the rate of activity will increase up to an optimum, and then decrease through heat stress. Thus, the relative position of these thresholds between the target pest and its intended control agent and differences in the rates of increase in activity will determine, at least in part, the efficacy of the control.

Establishment and activity are therefore at opposite ends of the 'thermal spectrum', the first being a large scale macrophysiological process and the latter governed by more local climatic conditions – but both can be quantified under current climatic patterns, and from such data, informed predictions can be made on how such ecophysiological attributes might be modified by different scenarios of climate warming. This paper reports on recent developments in methods to assess establishment potential and activity thresholds.

Establishment.

The recent focus for studies on establishment has been closely linked to the development of methods for the environmental risk assessment (ERA) of non-native

species, particularly in Europe. The regulatory framework in Europe is fragmented with some countries having stringent conditions for the import and release of non-native species whilst other countries, sometimes direct neighbours, have no restrictions. Among those countries with regulation, there has been a move toward a more evidenced-based risk assessment, characterized by the requirement to generate data from experimentation when such information is not available in the published literature. At the same time, and in recognition of the cost implications for small companies, van Lenteren *et al.* (2006) have proposed that risk assessment should have a hierarchical structure, whereby candidate agents are filtered through a series of tests, such that ‘risky’ species with little or no prospect of licensing are eliminated at an early stage, allowing resources to be channeled into the most promising agents (Fig 1).

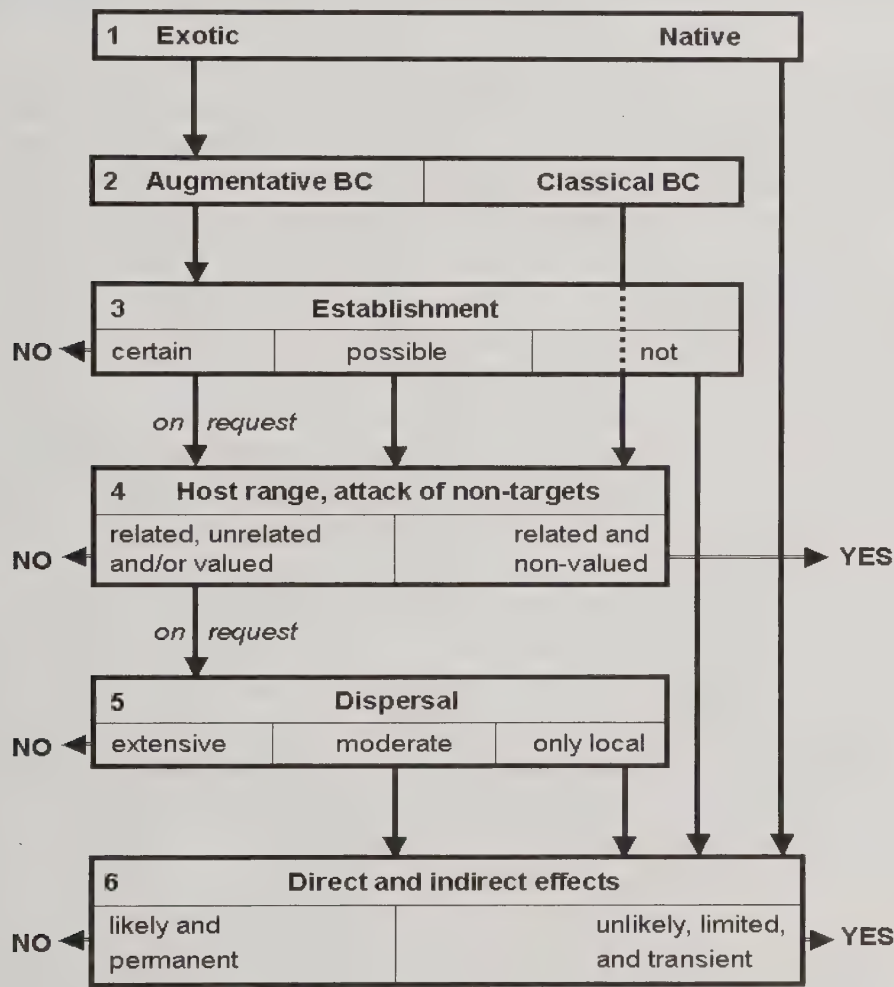


Fig. 1: Flow chart summarising a hierarchical environmental risk assessment scheme for arthropod biocontrol agents (van Lenteren *et al.*, 2006).

Whilst this approach has advantages for both regulatory authorities and companies, it is dependent on the development of reliable methods, which are either not yet available, in need of refinement, or too costly for industry. Importantly, a reliable method of predicting establishment is equally relevant to both classical and inundative glasshouse control. The first part of this paper therefore reports on

laboratory and field studies on the establishment potential of a number of glasshouse biocontrol agents (released or under consideration for release) in the UK.

Activity.

All insects and mites (pests and natural enemies) have temperature thresholds below which they are unable to develop or move, and in the case of winged insects, fly; and similar effects are seen at high temperatures. Some generalizations can be made about these various thresholds: thus, temperate species usually have lower developmental and activity thresholds than tropical species (Bale & Walters 2001), and can lower these thresholds after a period of low temperature acclimation (Powell & Bale 2008), whereas this is less common in tropical species. Also, for most species, there is a larger temperature differential between the chill coma and lower lethal temperature than between the heat coma and upper lethal temperature.

Various methods are available to determine the activity thresholds of insects and mites, but in general, they require 'direct observation' of the organisms as the temperature is increased or decreased. This paper describes a new method of video recording which allows retrospective analysis of data, and has applications in the areas of host range, prey preferences and efficacy.

MATERIALS AND METHODS.

Establishment.

The low temperature tolerance of various life cycle stages of eight non-native biocontrol agents with and without acclimation and in fed and starved conditions was assessed by a range of indices including the freezing temperature (supercooling point or SCP), lethal temperature (often higher than the freezing temperature), and lethal time (at -5°, 0° and 5°C). The same life cycle stages and treatment groups were also exposed outdoors (with moisture and protection from direct sunlight) and replicate samples retrieved from the field at intervals in winter. Temperatures experienced by the organisms were monitored continuously with dataloggers. The data from the laboratory and field exposures were analysed to identify laboratory indices that were the most reliable predictors of field survival (for full details of species, methods and results see Hatherly *et al.* 2005).

Activity.

The system comprised of an aluminium block within which a cooling fluid (antifreeze solution) was circulated from a low temperature programmable alcohol bath. An arena was milled into the aluminium block and covered by a thin sheet of clear perspex under which the insects or mites were contained. The activity of the organisms was filmed with a digital video camera (with macro lens) linked to a computer with customized software. A thermocouple is positioned 1mm above the base of the arena and coupled to a digital thermometer so that activity and temperature were recorded simultaneously. A set of interchangeable blocks with different arena diameters and depths enabled experiments to be conducted with organisms of different sizes ranging from microarthropods such as mites to larger species such as predatory mirids. For any selected species, comparisons could be

made between different life cycle stages and treatments (e.g. acclimated, starved or fed). A permanent record of each experiment is stored electronically, allowing retrospective visualization and analysis of a range of thermal tolerance traits. For small species, the arena can accommodate sample sizes of up to 40 specimens, each of which can be investigated individually by 'play back' of the recorded images (for a full description of the system see Hazell *et al.* 2008).

In this paper example data is presented on non-acclimated adults of the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) and the mirid *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae). To determine the walking speeds of *A. swirskii*, 20 mites were placed in a 25 mm diameter by 7.5 mm deep arena and cooled from 30°C at 0.5°C min⁻¹ to 25°, 20°, 15° and 10°C and held at each of these temperatures for 5 min after which the walking speeds were measured. The chill coma temperature of *A. swirskii* was assessed by cooling two samples of 20 mites from 25°C to 10°C at 0.5°C min⁻¹ and then from 10° to -10°C at 0.1°C min⁻¹. In the context of these experiments chill coma is defined as the temperature at which an individual ceases to move i.e. walk. At a lower temperature, the organism will cease movement of all appendages (antenna, leg), and this value is also sometimes described as the chill coma, or critical minimum temperature (CT_{min}) – see Hazell *et al.* (2008) for a discussion of these terminologies. With the mirid *N. tenuis*, 10 specimens were placed in a 40 mm diameter by 7.5 mm deep arena at their culture temperature (23°C) and cooled at 0.5°C min⁻¹ to 20°C and then at the same rate to 0°C at intervals of 2.5°C. At each 2.5°C interval, the temperature was held for 5 min and the activity recorded at these temperatures. For the chill coma experiments, mirids were placed in the same arena at their culture temperature and the temperature reduced at 0.2°C min⁻¹ to -15°C. The video recordings for both experiments were then analysed to generate the data.

RESULTS.

Establishment.

A series of earlier analyses on five non-native (to the UK) control agents: *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae), *Macrolophus caliginosus* Wagner (Hemiptera: Miridae), *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae), *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) and *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae) identified the lethal time that killed 50% of sample populations (LTime₅₀) at 5°C to be strongly correlated with survival time in the field in winter (Hatherly *et al.* 2005). Further data has since been acquired on the mirids *Dicyphus hesperus* Knight (Hemiptera: Miridae) and *N. tenuis* and the mite *A. swirskii* and the same correlation has been substantiated (Fig. 2). All field experiments were carried out at the University of Birmingham (Birmingham, UK).

There is a strong correlation between survival in the laboratory at 5°C and under variable winter temperatures in the field, and no species has yet been studied that departs from this trend. Given the importance of possible outdoor establishment in northern Europe of glasshouse biocontrol agents, this relationship is important for two reasons. Firstly, it represents a method that can be incorporated into the environmental risk assessment proposed by van Lenteren *et al.* (2006). Secondly,

the experiments could be conducted by companies within their own research facilities. The important consideration here is whether the ‘predictive relationship’ across these eight species is now sufficiently robust that laboratory assessment alone could be used to assess establishment potential.

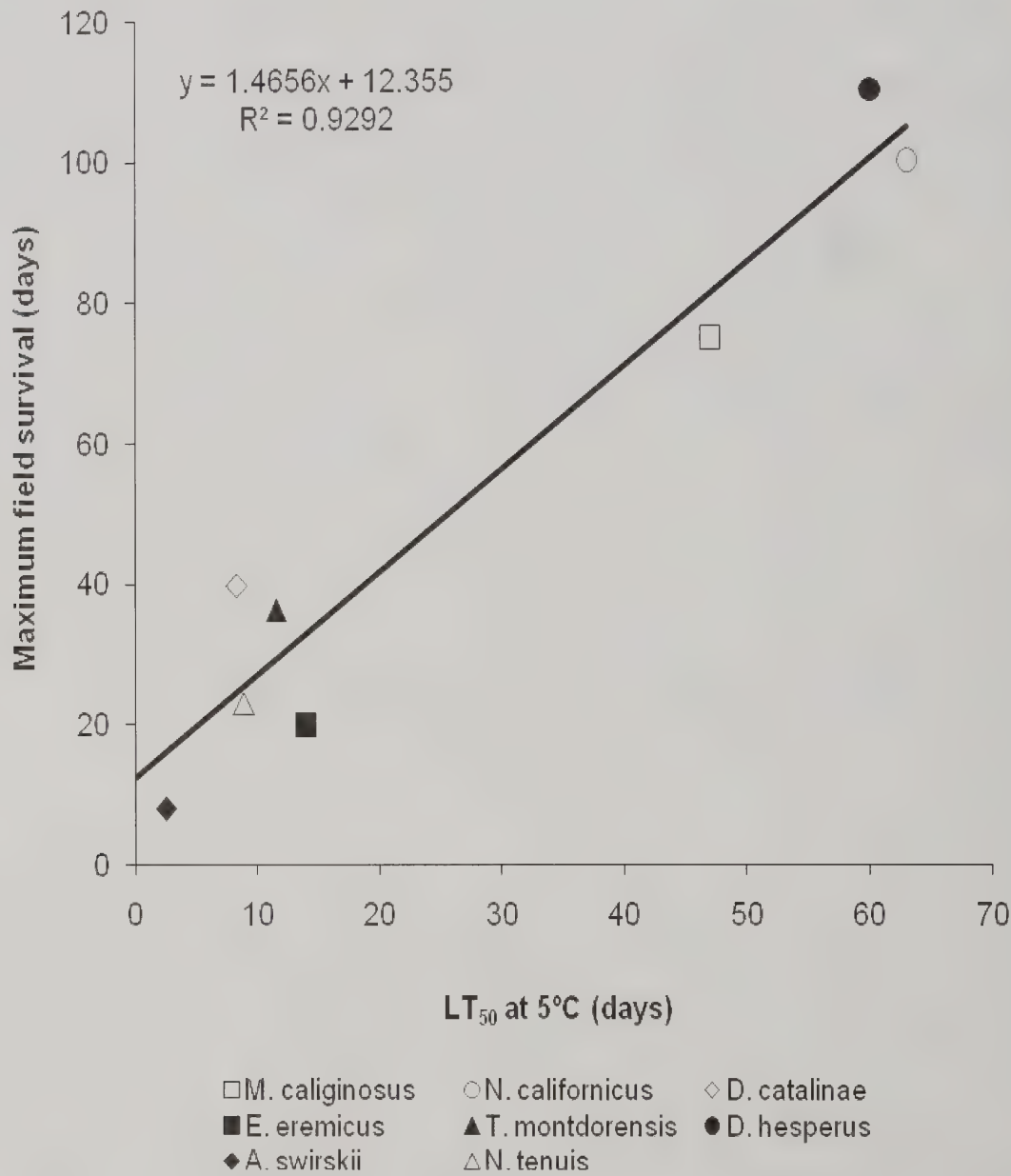


Fig. 2. Relationship between the $LTime_{50}$ at 5°C (days) and maximum field survival in winter of eight biocontrol agents non-native to the UK (see text for details)

Activity.

Data on the walking speeds at 20° and 10°C and chill coma temperature of *A. swirskii* and *N. tenuis* are shown in Table 1.

Table 1. Walking speeds and chill coma temperature of non-acclimated adult *A. swirskii* and *N. tenuis*.

	<i>A. swirskii</i>	<i>N. tenuis</i>
Walking speed (cm min ⁻¹) ± SE		
20°C	20.8 ± 1.4	31.0 ± 1.8
10°	7.1 ± 0.5	8.9 ± 1.6
Chill coma (°C) ± SE	6.2 ± 0.1	3.2 ± 0.1

The data on *A. swirskii* and *N. tenuis* should not be regarded as directly comparable because the species could be used in different biocontrol situations. However, the data do show that whilst the mobility of both species is greatly reduced at 10° compared with 20°C, they are still active at the lower temperature. Below 10°C, *N. tenuis* is still capable of some movement at a temperature at which *A. swirskii* has become inactive. This method provides an effective measure of the temperature-mobility interaction which is an important component in the efficacy of biocontrol, particularly with regard to predators. As such, in a screening programme for relative efficacy, this system can provide valuable data, especially when the mobility of candidate agents is compared with that of their prey.

DISCUSSION.

The focus on the establishment potential and wider environmental risks of non-native biological control agents is a paradox, as indicated by van Lenteren *et al.* (2006). In the long history of biocontrol, with numerous introductions of a large number of species in many parts of the world, there have been remarkably few negative effects. And yet, there has been a trend toward greater regulation, with well organized systems in North America, Australia and New Zealand, and a move toward a more coordinated approach in Europe. It is difficult to identify a single reason that explains these developments, but in general it represents the increasing emphasis on environmental protection within agricultural systems, and a greater awareness of possible threats to native species and ecosystems – the recent expansion through Europe of the predatory ladybird *Harmonia axyridis* being a prime example. Whilst the biocontrol industry has to work within this framework, it is also important that the regulatory requirements are proportionate to the risks (real rather than perceived), and affordable by companies with limited R&D budgets.

The hierarchical risk assessment proposed by van Lenteren *et al.* (2006) has the dual merits of identifying ‘risky species’ early in the process, thus allowing companies to focus resources on the most promising candidate agents. However, the ERA is dependent on having reliable methods and where possible, techniques that industry can implement within their own facilities. For releases into glasshouses in temperate and colder climates a case can be made for assessing establishment

potential as the 'first test', because if it can be shown that a species would be unable to survive for more than 2-3 weeks in winter in the outdoor environment, there would be no prospect of wider establishment, and there would be no need to invest in host range tests that can be expensive. However, if the release was to be made for instance, in southern Europe, the climate would support year-round development and reproduction, and host range tests would then be essential. For those species where risk of establishment is the crucial factor in the application for a release permit, the studies on 8 natural enemies across a range of taxa suggest that there is a very strong correlation between laboratory and field survival at low temperature (Fig. 2). The absence of any exceptions to this relationship suggests that a laboratory assessment alone (at 5°C) may now be an adequate basis for assessing establishment potential. It then becomes relevant to ask whether data obtained in a UK study be applied to other countries, and how climate warming might impact on this relationship. By reference to climate records for other European countries (or parts thereof) and in other parts of the world, it would be relatively straightforward to identify countries and regions where the climate is either colder (e.g. Scandinavia) or similar (The Netherlands, northern France and Germany) to the UK and to which the identified relationship should apply. The impacts of climate warming are more difficult to quantify. There are a number of examples of pest species extending their northern range margin by around 50 km over the past 30 years (see Introduction) against a backdrop of a ~1°C increase in mean or minimum temperatures over the same time period. The general implication is that climate warming may promote enhanced winter survival, but whether this would allow species currently regarded as 'safe' (see Fig. 2) to become capable of establishment in a temperate or colder climate seems doubtful, unless the level of temperature increases were toward the maximum end of current predictions.

The technique described for assessing activity and chill coma has some major advantages over previous systems: cooling regimes are flexible and programmable, there is an accurate 'real-time' visual display of temperature that is directly relatable to the observed insect behaviours, and every experiment produces a permanent record that can be analysed and re-analysed as and when required. The method enables the activity and chill coma temperatures of biocontrol agents to be directly compared, as well as that of their target prey, which is relatable to both glasshouse climatic regimes and outdoor conditions. The system also has applications in studies on predator-prey interactions, including variations such as relative densities and the influence of host plant species.

In summary, temperature has both direct and indirect effects on biocontrol agents, their target prey and hosts, and the wider ecosystem within which these organisms interact. These effects vary in scale from the macrophysiological perspective of distributions and abundance to localized effects on development, reproduction and activity. In order for the potential of biocontrol to be more fully realized, it is necessary to develop reliable methods to assess both the environmental risks of novel agents, and the factors that might affect their efficacy, of which climate, and particularly temperature, is clearly important. The techniques described and evaluated in this paper could be immediately utilized by researchers and companies and contribute toward the overall aim of releasing safe and effective biological control agents to combat the increasing problems caused by arthropod

pests, which are likely to increase and become more widespread in an era of climate change.

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THE DISTRIBUTION OF *MICROCTONUS HYPERODAE* AND *M. AETHIOPOIDES* (HYMENOPTERA: BRACONIDAE) IN NEW ZEALAND

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ABSTRACT.

Two species of the parasitoid wasp in the genus *Microctonus* (Hymenoptera: Braconidae) have been successfully introduced to New Zealand as biological control agents of weevil pasture pests. *Microctonus hyperodae* Loan was introduced against *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) and *M. aethiopoides* Loan against *Sitona discoideus* Gyllenhal and *S. lepidus* Gyllenhal (Coleoptera: Curculionidae). The strain of *M. aethiopoides* that attacks *S. discoideus* has successfully established throughout New Zealand and has effected significant reduction in damaging populations of its host. The strain of *M. aethiopoides* that attacks *S. lepidus* has also been successfully introduced to Waikato, Hawkes Bay and Nelson, but has not established in Northland. Developmental data and known locality data can be used to construct CLIMEX models for *M. hyperodae* and both strains of *M. aethiopoides* to predict their New Zealand distribution. The potential geographic ranges of the parasitoids are compared and the results used to indicate how this information could be applied to future parasitoid releases.

TOO HOT TO HANDLE? RANGE EXPANSION AND LIFE-HISTORY RESPONSES TO CLIMATE WARMING OF THE SOUTHERN GREEN STINK BUG *NEZARA VIRIDULA* (HETEROPTERA: PENTATOMIDAE)

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ABSTRACT.

We compared the past and the current limits of the distribution range of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae), in central Japan. In the early 1960s, the northern limit of the range was in Wakayama Prefecture and was limited by a +5 °C isothermal line for the mean January temperature. In 2006–2007, a new survey demonstrated that this northern limit had shifted northward by 85 km, at a mean rate of 19.0 km/decade. The shift was likely promoted by milder thermal conditions in winter.

The effect of simulated climate change on *N. viridula* was further studied close to the species' northern range limit. Insects from the same egg masses were reared for 15 months in 10 consecutive series under quasi-natural (i.e. outdoor) conditions and in a transparent incubator, in which climate warming was simulated by adding 2.5 °C to the outdoor temperature. The warming strongly affected all life-history and phenological parameters. In the spring, simulated warming advanced the timing of body colour changes and post-diapause reproduction. In the early summer, it increased egg production and accelerated nymphal development. In the late summer (the hottest season), the effect of the simulated warming was strongly deleterious: nymphs developed slowly, suffered higher mortality and had difficulties during final moulting; the emerged females were smaller, some exhibited abnormal cuticle, produced fewer eggs and had a decreased life span. In the autumn, the warming accelerated nymphal development, resulted in larger female size, affected the timing of the diapause-associated adult body colour change from green to russet and enhanced preparation for overwintering. Larger females had a higher winter survival rate than smaller females. The warming strongly increased survival rate in both size classes and allowed smaller females to reach the same winter survival rate as larger females had under the quasi-natural conditions. The winter survival also differed between the green and dark-coloured females under the quasi-natural, but not under the warming conditions. However, under the warming conditions, green females survived the winter even better than dark-coloured females did under the quasi-natural conditions. Moreover, the warming shortened the life span of females from the summer generations and prolonged it in those from the autumn generation. It is concluded that even a moderate temperature increase (+2.5 °C) in the future is likely to have a complex influence upon insects, strongly affecting many of their life-history and phenological parameters.

INTRODUCTION.

Rapid climate change implies simultaneous and complex changes of many environmental variables – primarily air temperature (IPCC 2007). There is now considerable attention directed to how the global biota are and will be responding to global warming (Bale *et al.* 2002; Deutsch *et al.* 2008; IPCC 2007; Parmesan 2006).

The southern green stink bug, *Nezara viridula*, is apparently responding rapidly to climate warming (Musolin 2007; Musolin *et al.* 2009; Tougou *et al.* 2009; Yukawa *et al.* 2007). The species occurs in an ever-widening range throughout tropical and subtropical regions and Japan is at the northern margin of *N. viridula*'s Asian range. In the north, *N. viridula* is replaced by a congeneric species, the oriental green stink bug, *Nezara antennata* Scott. Both species, and especially *N. viridula*, are major agricultural pests.

In the early 1960s, Kiritani and co-workers conducted a wide-scale field survey and mapped the northern limit of the range of *N. viridula* in central Japan (Fig. 1a; Kiritani *et al.* 1963). They showed that the northern limit of *N. viridula*'s distribution in central Japan occurred in Wakayama Prefecture (latitude approximately 34.1°N). *Nezara antennata* dominated in northern and central parts of the prefecture, whereas coastal and the southern parts of the prefecture were mostly or completely occupied by *N. viridula* (Fig. 1a). In general, *N. viridula* was found to occur sympatrically in warmer parts of the distribution range of *N. antennata*. The area of co-existence of two species was shown to lay on the +5 °C isothermal line for mean temperature of the coldest month (usually January) and, thus, January temperature was proposed as the principal factor determining the northern limit of *N. viridula*'s distribution (Kiritani *et al.* 1963).

In our attempt to understand and predict how insects will respond to further warming, we: (1) compared the past and the current limits of the distribution range of *N. viridula* in central Japan; and (2) studied experimentally the effect of simulated global warming on life-history traits and phenology of *N. viridula* by exposing two groups of insects for a 15 month period to outdoor conditions and conditions simulating a moderate temperature increase (2.5 °C), which is within the likely range of temperature increase (1.1–6.4 °C) predicted for the end of the XXI century (IPCC 2007).

MATERIALS AND METHODS.

Range Change Survey.

To determine the current northern limit of *N. viridula*, an extensive field survey was conducted in six prefectures in central Japan in 2006–2007 (Tougou *et al.* 2009).

Phenology and Life-history Responses to Simulated Climate Change.

Adults of *N. viridula* were collected in Kochi (33.6°N, 133.6°E) and transferred to Kyoto (35.0°N, 135.8°E), where the experiment was conducted. On ten dates in 2006–2007 (i.e. 1 June, 1 July, etc.), egg masses were transferred to plastic containers and placed under two experimental conditions: *quasi-natural* (i.e. *outdoor*) and *simulated warming conditions*. Under the quasi-natural conditions, insects were

reared in plastic containers on metal shelves open from all sides. Under the simulated warming conditions, insects were reared similarly in a specially modified incubator LH-350NSZ (NK Systems, Osaka) placed next to the open shelves. The incubator had transparent glass walls to ensure the insects experienced the same photoperiodic conditions that their outside siblings did. A sensor measured outdoor temperature on the shelves of the quasi-natural conditions and the incubator constantly simulated warming conditions inside by adding 2.5 °C to the outside temperature. In each series and each treatment, all important events such as hatching, moulting, copulation, oviposition, adult colour change, and death were recorded daily. Only data on females are presented. Life-history and phenological parameters were compared between the two conditions and all series to examine whether and how simulated warming affected performance and fitness of *N. viridula* (Musolin *et al.* 2009).

RESULTS.

Range Change Survey.

The survey of 2006–2007 demonstrated that the northern limit of *N. viridula*'s distribution range had shifted northward by 85 km from the early 1960s (Fig. 1).

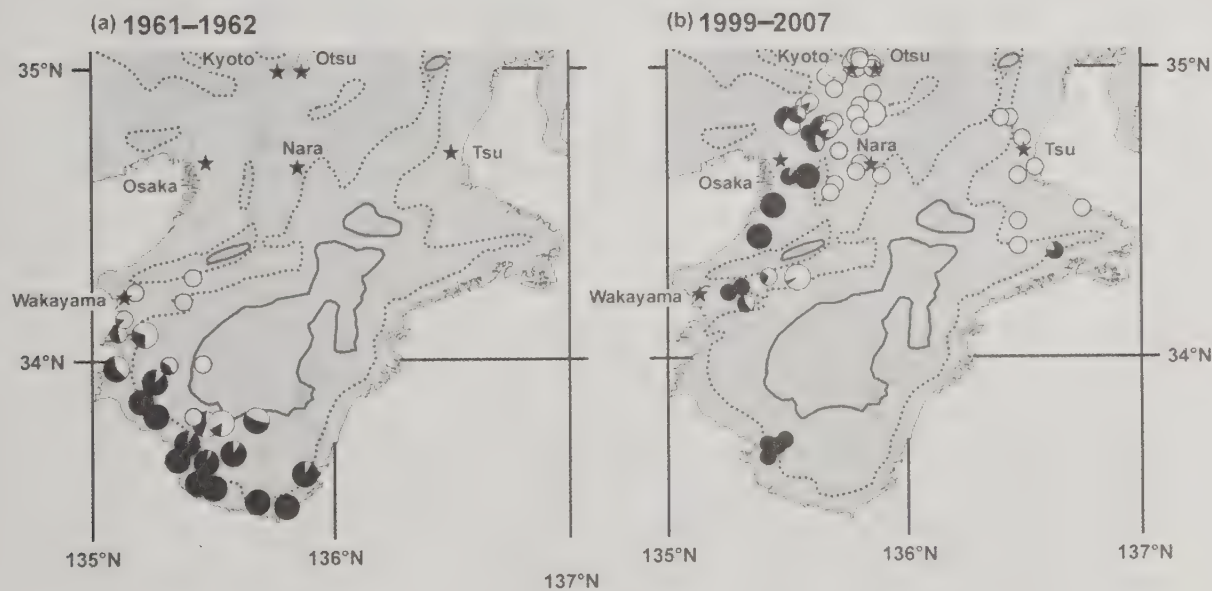


Fig. 1. Distribution and relative abundance of *Nezara* spp. in central Japan: (a) in the early 1960s (Kiritani *et al.* 1963); and (b) in 2007 (Tougou *et al.* 2009). Symbols: black sections, *N. viridula*; white sections, *N. antennata*. Sample size: small circles, 1–50 specimens; large circles, more than 50 specimens. Elevation: dotted line, 500 m above sea level; solid black line, 1,000 m above sea level.

PHENOLOGY AND LIFE-HISTORY RESPONSES TO SIMULATED CLIMATE CHANGE.

Nymphal development. The duration of the nymphal period showed a predictable seasonal trend under the quasi-natural conditions: higher outdoor temperatures in August resulted in a shorter nymphal period in the 1 August series compared to the 1 June and 1 July series and as the season progressed the nymphal period became significantly longer (Fig. 2). The effect of the artificial warming was unexpected. While warming significantly accelerated nymphal development in the 1 June, 1 and 15 September series, it retarded development to varying levels in the 1 July and 1 August series. Thus, while under the quasi-natural conditions the nymphal development tended to be faster in the 1 August series than in the June and July series, under warming conditions it tended to be slower.

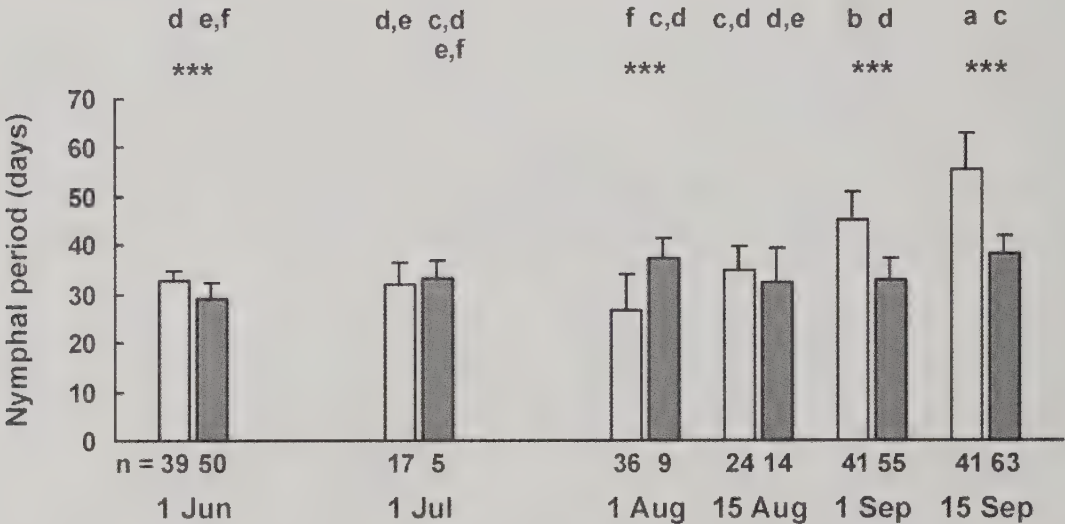


Fig. 2. Mean duration (+SD) of the nymphal period in females of *Nezara viridula*. Bar colour: white, the quasi-natural conditions; grey, the simulated warming conditions. Stars: pair-wise comparison between the two treatments within the same series, *t*-test; ***, $P < 0.001$. Letters above bars: multiple comparison (all series and treatments together), Tukey–Kramer HSD test; means with the same letters are not significantly different ($P > 0.05$) (from Musolin *et al.* 2009).

When the rate of nymphal development was calculated, averaged for the series and plotted against the mean temperature experienced during the nymphal period, most data points fit well to a linear regression line, although three data points corresponding to the warmest season (the 1 July, 1 and 15 August series) were below and far from the line (Fig. 3).

The simulated warming also affected survival of nymphs. In summer it led to increased nymphal mortality, some adults in these series had apparent difficulties while moulting to the adult stage and many of them died during moulting.

Size and physical condition of adults. The body length of females tended to be smaller in the 1 and 15 August series than in most of other series (Fig. 4). The simulated warming strongly enhanced this tendency. When compared pairwise with

the quasi-natural conditions on the same dates, warming significantly reduced the size of adults in August, but increased it in September.

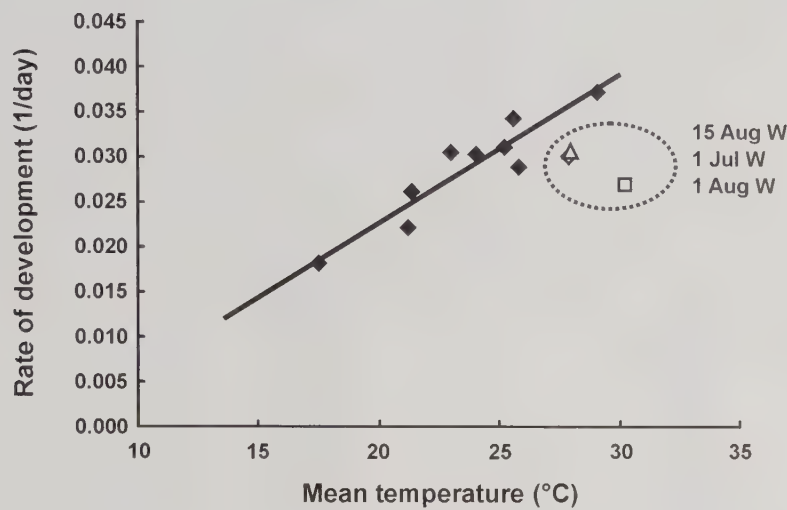


Fig. 3. Effect of temperature on the rates of nymphal development in females of *Nezara viridula*. The linear regression line ($E_{(1,8)} = 47.937$, $P = 0.0002$; ANOVA) is drawn for all series and treatments except three encircled mid-summer series under the simulated warming conditions (from Musolin *et al.* 2009).

In addition to having a smaller size in the warmest season, some adults were apparently in a weaker physical condition under the warming conditions: they had a softer cuticle and a lighter yellowish body colour instead of intensive green.

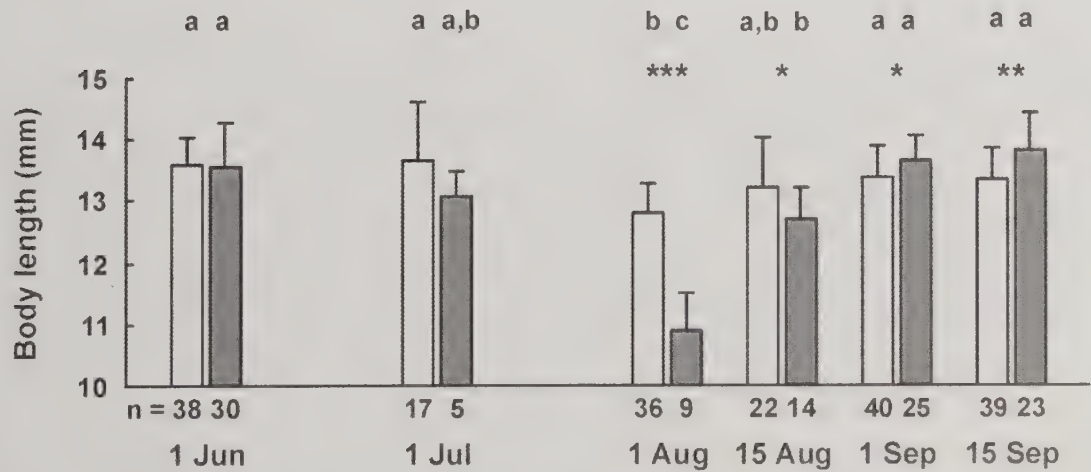


Fig. 4. Mean (+SD) body length of *Nezara viridula* females. Bar colour: white, the quasi-natural conditions; grey, the simulated warming conditions. Stars: pair-wise comparison between the two treatments within the same series, *t*-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Letters above bars: multiple comparison, Tukey–Kramer HSD test; means with the same letters are not significantly different ($P > 0.05$) (from Musolin *et al.* 2009).

Adult size and winter survival. Under both conditions, the series with larger mean body length tended to have a higher winter survival than those with smaller means (Fig. 5a), although, this trend was not statistically significant likely because of the very low survival rate in some series under the quasi-natural conditions. However, when analysed on an individual basis, both the female size and the simulated warming strongly affected the success of overwintering. Larger females had significantly higher winter survival rates under each of the two experimental conditions ($P \leq 0.008$; Fig. 5b). Moreover, females from each size group had higher winter survival rates under the simulated warming conditions than under the quasi-natural conditions ($P \leq 0.06$; Fig. 5b). Finally, the simulated warming allowed the smaller females to reach the same winter survival rate as the larger females had under the quasi-natural conditions.

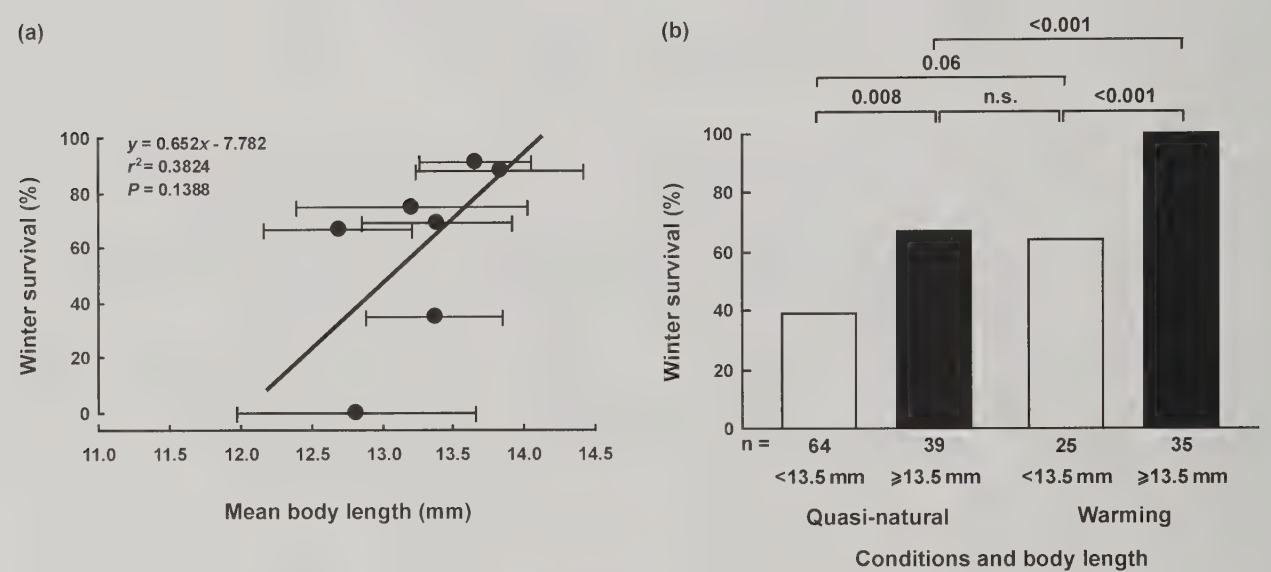


Fig. 5. Effect of body size on winter survival rate of *Nezara viridula* females: (a) The relationship between winter survival and mean (\pm SD) body length. All series and treatments in which females survived until at least 1 December are shown, the linear regression line and statistics after arcsine transformation are shown; (b) Winter survival rate in different body length groups of non-reproductive females under different treatments (all series are combined; P of Fisher exact test after Bonferroni adjustment is shown) (from Musolin *et al.* 2009).

Adult body colour and winter survival. Induction of winter diapause in *N. viridula* is associated with adult body colour change from green to russet. The experiment showed that winter survival of females was strongly correlated with their body colour. When winter survival was analysed separately for each colour grade in the non-reproductive females, it differed significantly between green and russet females, but not between each of those colour grades and the intermediate colour grade (Fig. 6a). The simulated warming strongly influenced the relationship between body colour and winter survival. Under the quasi-natural conditions the dark-coloured females had a significantly higher survival rate than the green females ($P < 0.001$), whereas under the warming conditions the survival rates were higher in both colour grade groups

and did not differ significantly (Fig. 6b). The comparison of winter survival within each colour grade but between the conditions showed that the green females had a significantly higher survival rate under the simulated warming conditions ($P = 0.01$). Survival of the dark-coloured females was also higher under the warming conditions, although insignificantly (Fig. 6b).

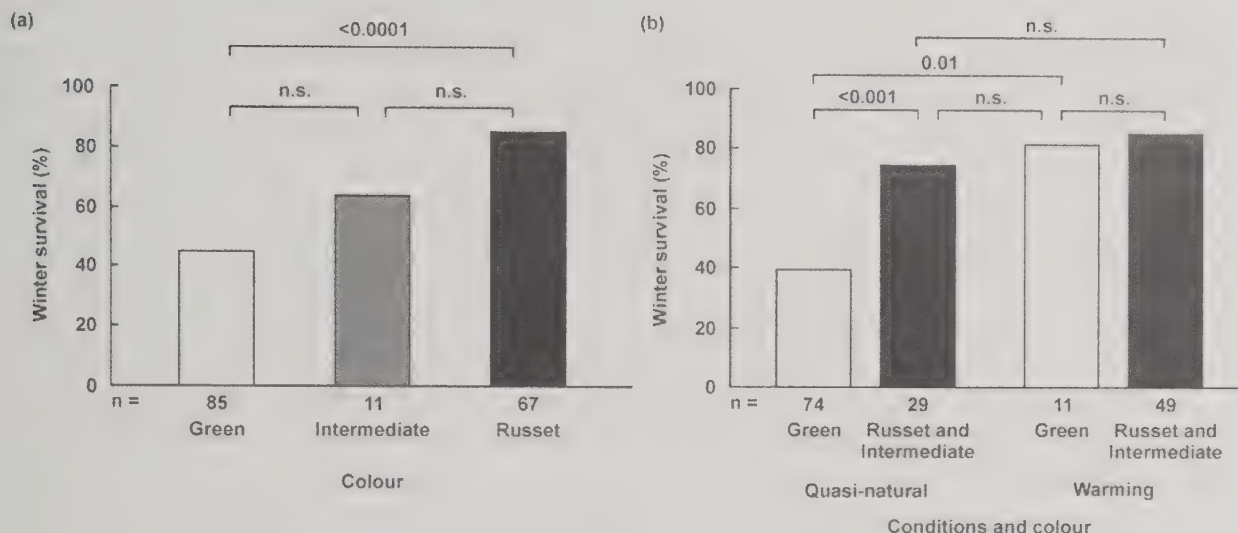


Fig. 6. Effect of colouration on winter survival of *Nezara viridula* females. Winter survival in: (a) different colour groups of non-reproductive females (all series and treatments combined; P of χ^2 test is shown); and (b) different colour groups of non-reproductive females and under different treatments (all series are combined; P of Fisher exact test is shown) (from Musolin *et al.* 2009).

Timing of reproduction. Before overwintering, females tended to start both copulation and oviposition earlier under the simulated warming than under quasi-natural conditions, although the difference was not significant likely due to the small number of reproducing females under the warming conditions. In the hottest mid-summer season, the start of reproduction tended to be delayed. After overwintering, the trend to advance reproduction was more pronounced: in all the series, copulation and oviposition started earlier under the simulated warming conditions than under quasi-natural conditions and in most cases the difference was statistically significant.

Fecundity. In the early summer, the simulated warming significantly increased egg production, whereas later in the season fecundity tended to be lower under the simulated warming than under the quasi-natural conditions, although the difference was not significant. After overwintering, no common patterns were detected and only in the 15 September series did the warming significantly increase post-diapause egg production.

Longevity. The simulated warming significantly reduced longevity of females in the summer series ($P < 0.001$). In the autumn series, most females survived until the next spring and early summer and the simulated warming increased their longevity likely by optimizing thermal overwintering conditions.

DISCUSSION.

Range Change.

The survey demonstrated that the northern limit of *N. viridula*'s distribution had shifted northward by 85 km from the early 1960s to 2006–2007, at a mean rate of 19.0 km/decade (Fig. 1). Analysis of climatic data shows that the mean January–February temperature in the region was 1.03–1.91 °C higher in 1998–2007 than in 1960–1969. The number of cold days (with the mean temperature below +5 °C) also significantly decreased, while the annual lowest temperature significantly increased. *Nezara viridula* was found mostly close to those locations where: (1) the mean January temperature exceeded +5 °C, (2) the mean number of cold days did not exceed 26 in January–February, and (3) where the mean annual lowest temperature did not drop below –3.0 °C. The general linear model shows that the mean January temperature and number of cold days are the most important factors controlling the northern limit of distribution of *N. viridula*. Altogether, the climatic data suggest that over the last 45 years environmental conditions have become more favourable for overwintering of *N. viridula* at many locations in central Japan. This has likely promoted the northward spread of the species, representing a direct response to climate warming. A sympatrically distributed congeneric *N. antennata* seems to respond to the warming by a retreat from the ocean coast towards cooler elevated habitats, which might be a complex response to elevated temperature and interspecific mating with *N. viridula* (Musolin *et al.* 2009).

Phenology and Life-history Responses to Simulated Climate Change.

As in many species (Parmesan 2006), temperature elevation is expected to affect numerous phenological events in *N. viridula*. For example, in this experiment, the simulated warming advanced the start of the spring colour change and reproduction. A further temperature increase is likely to stimulate an earlier start of reproduction in *N. viridula* and perhaps other insects, provided that they can find enough food.

The simulated warming strongly affected the timing of nymphal development. The nymphal growth was significantly accelerated by the warming in the early summer and autumn series, but retarded in the late summer series (Fig. 2). The late summer is the warmest period in central Japan: in August 2006, daily maximum temperature reached +40.0 °C under our experimental quasi-natural conditions and, thus, +42.0 °C under the simulated warming conditions. The relationship between the rate of nymphal development and temperature (Fig. 3) further shows that the simulated warming made the mid-to-late summer conditions unfavourable for nymphal development. As a result, nymphs developed slower, suffered higher mortality, and had difficulties during the final moulting.

The simulated warming also affected many life-history traits. In general, under the quasi-natural conditions, females of *N. viridula* had a smaller body size in the 1 August series than earlier in summer or later in autumn (Fig. 4), a seasonal tendency that has been reported in some other insect species. The simulated

warming strongly enhanced this tendency: females that emerged in the 1 and 15 August series were not only the smallest in the entire experiment, but on each of these dates females were significantly smaller under the simulated warming conditions than under the quasi-natural conditions. These findings show that the response to further warming will not be the same through out the year and will differ by season. Difference in size, in turn, is likely to affect other life-history traits such as survival or reproductive performance.

Winter is a critical season for insects in the temperate zone. Under both experimental conditions, larger females had significantly higher winter survival rates than smaller females (Fig. 5). More importantly, an additional 2.5 °C of the simulated warming during the winter strongly increased the likelihood of winter survival in both size classes and allowed smaller females to reach the same winter survival rate as larger females had under the quasi-natural conditions. Thus, if warming continues in the future, not only larger but also smaller females of *N. viridula* will survive winters more successfully and this will likely provide a basis for faster population growth.

The simulated warming strongly affected the functional relationship between the colour change in autumn and overwintering success. Under the warming conditions green females survived the winter even better than dark-coloured females did under the quasi-natural conditions (Fig. 6). This finding indirectly implies that the further climate warming will increase chances of successful winter survival even in those individuals that failed to change body colour and, thus, properly prepare for diapause before the winter.

Whereas it is believed that many species in the temperate zone will benefit from the temperature rise in one or another way (e.g., Deutsch *et al.* 2008), the present experiment shows that the situation is more complicated. The strong retardation of nymphal development, smaller size, softer cuticle and lighter yellowish body colour of *N. viridula* adults as well as their dramatically reduced life span in the 1 August series suggest that the elevated temperature experienced by nymphs and young adults exceeded their thermal optima. The mechanism underlying the abnormality of the cuticle colour and structure is so far unknown, although it might be related not only to thermal stress experienced by the bugs but also to the malfunction of their gut symbiotic bacterial fauna caused by the daily high temperature extremes (T. Fukatsu, pers. comm.). The design and the results of our experiment suggest that the warming-mediated suppression of performance might be a direct effect of temperature rather than an indirect one (via altered precipitation or condition of host plants). On the other hand, in contrast to plants, active stages of insects can possibly minimize the deleterious effect of elevated temperature, for example, by active selection of microhabitats. This might be especially useful in coping with daily temperature extremes. Some insect species will probably be able to mitigate the negative hot season effects of warming by evolving a summer diapause.

The data accumulated so far suggest that the effect of the rapid climate change is likely to be complex and differ among species and regions. The current study demonstrates that, even within the same species or population, responses will be different for different life-history traits and seasons. Thus, for instance, warming might negatively affect nymphal development during the hot season, stimulate development in autumn and/or strongly enhance survival of adults in winter. All this together, in a

complex way, will affect a species population dynamics, voltinism, relationships with other members of the community and likely pest status.

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IMPROVEMENT IN THE PRACTICAL USE OF AN APHIDOPHAGOUS GALL MIDGE, *APHIDOLETES APHIDIMYZA* (DIPTERA: CECIDOMYIIDAE), IN GREENHOUSES

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ABSTRACT.

To make natural enemies fully exert their potential, we have to provide them with favourable conditions for their performance. The predacious gall midge *Aphidoletes aphidimyza* is distributed worldwide and has been used in many countries as one of the most effective biological control agents against aphids in greenhouses. Since 1998, mass-produced cocoons of *A. aphidimyza* have been imported to Japan from The Netherlands. However, we additionally studied ecological and behavioural traits of the predator to improve practical methods of releasing *A. aphidimyza*. The objectives of our work were to find measures to (1) minimize the failure of adult emergence from cocoons buried in vermiculite, (2) increase mating rates by providing adult midges with artificial mating sites, (3) prolong adult longevity, (4) increase fecundity, and (5) avoid the effects of 'jet lag' when cocoons are imported from The Netherlands. In addition, continuous importation of European populations has been causing concern because of possible genetic and ecological contamination of native Japanese populations with European ones. We refer to this issue on the basis of molecular data and the results of cross mating between the different populations. Moreover, aphids have a great potential to increase the number of annual generations under global warming because of their low developmental zero point and low amount of effective cumulative temperature required for one generation. Therefore, more attention has to be paid to aphid control in greenhouses as a model case of global warming.

INTRODUCTION.

To make natural enemies fully exert their potential, we have to provide them with favourable conditions for their performance. The predacious gall midge, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae), is distributed worldwide including Japan, and larvae of this species feed on a wide variety of aphids, at least 80 species having been recorded as hosts (Yukawa *et al.* 1998). Unlike other gall-inducing species of Cecidomyiidae, larvae live freely on plants and search actively for aphids. Full-grown larvae drop to the ground, burrow their way through the soil to a depth of about few to 30 mm, and secrete silk cocoons. Larvae pupate in the cocoons within a few days and adults emerge after 1-3 weeks. Before emergence, pupae come out their cocoons and move up to the soil surface.

Mating and egg laying occur as light intensity falls at dusk (Harris 2004). At the time of mating, males and females hang on abandoned spider-webs or other substrates in a face-to-face position (Hauser 1986; Schelt 2000). Both males and females take aphid honeydew as an energy source (Harris 1973). The life span of males is always shorter than that of females. Adult life span was significantly extended if the adults were supplied with honeydew or sugar solution (Kuo 1985). Eggs are laid singly or in clusters of up to 40 eggs and they are usually placed on plants, near the aphid colonies (Harris 1973). Eggs are oval, about 0.3 X 0.1 mm, orange in colour and barely recognizable to the naked eye. They hatch about 3 to 4 days after laying and the first instars immediately seek out and attack aphids.

This gall midge has been used in many countries as one of the most effective biological control agents against aphids, particularly in greenhouses, and proved effective (e.g., Meadow *et al.* 1985; Morse & Croft 1987; Nijveldt 1988; Solarska 2004). In Japan, *A. aphidimyza* has been registered since April 1998 as a biological control agent, and mass-produced cocoons of *A. aphidimyza* have been imported from The Netherlands. Therefore, the opportunity for growers to use it has been increasing. However, as far as we know, no striking effects of *A. aphidimyza* on aphid control have been reported yet from Japan. Failure of successful usage in Japan might have been caused by a lack of detailed ecological or behavioral information on *A. aphidimyza*. In order to obtain adequate results in practical use, detailed ecological and ethological studies of *A. aphidimyza* are urgently needed.

For this purpose, the following ecological or behavioral traits of *A. aphidimyza* were investigated: **(1)** the survival of pupae in relation to the depth of pupation sites to minimize the failure of adult emergence from cocoons buried in vermiculite, **(2)** the importance of substrates for adults to hang from for successful mating, **(3)** the influence of nutrient on the longevity and fecundity of females, and **(4)** the hourly emergence pattern.

This paper reports the results of these investigations in order to provide favourable conditions for the performance of *A. aphidimyza*. We also refer to the possible genetic and ecological contamination of native Japanese populations with European ones on the basis of molecular data (Shirota *et al.* 1999) and the results of preliminary cross-mating tests between the different populations. In addition, we briefly mention possible effects of global warming on aphid populations.

MATERIALS AND METHODS.

The failure of adult emergence from cocoons buried in vermiculite.

In this study, mass-rearing cocoons produced by Koppert B.V., The Netherlands, for commercial application were imported through Arysta Life Science Corporation (Tokyo), and used for various experiments soon after they arrived in the laboratory because adults began to emerge within 1 to 3 days. These cocoons were kept in bottles with moist vermiculite when imported to Japan. The bottles were 190 mm in height and 65 mm in diameter. Before experiments, one of the bottles was selected, the cap taken off, and the bottle put into a rearing cage, which was kept in an incubator at 25°C under 12L:12D conditions for 3 days to record the number of adults emerging successfully from cocoons buried in the vermiculite layer. Then, the

failure of adult emergence was investigated by counting the number of cocoons containing dead pupae and the number of adults that died during emergence on the way to the surface of the vermiculite layer. Thereafter, the rate of successful adult emergence was investigated in plastic cylinders (250 mm in height, 70 mm in diameter) by burying 200 cocoons at different depths (150, 100, 50, and 10 mm) of vermiculite layer, respectively. Relatively high humidity was maintained by daily moistening filter papers on the bottom of the cylinders.

The importance of substrates for adults to hang on for successful mating.

Mating success was evaluated by comparing the numbers of eggs laid on strawberry plants between two kinds of treatment in rearing conditions. Into two plastic cylinders, 20 pairs of newly emerged adults of *A. aphidimyza* were released respectively and kept there for 24 h to give them opportunities for mating. In one of the cylinders, very fine fishing lines, instead of abandoned spider-webs, were strung between the inner sides of the cylinder, providing them with substrates for hanging on. Nothing was provided in the other cylinder. Then, 20 females were transferred from each cylinder to a new cylinder containing 2 potted strawberry plants infested with *Aphis gossypii* Glover (Hemiptera: Aphididae). The strawberry plants used in this experiment were almost the same size with a total of 18 leaves on six shoots and infested by roughly equal numbers of aphids. After 24 h, the number of eggs laid on the strawberry plants was recorded.

The influence of nutrient on the longevity and fecundity of females.

Newly emerged males and females were fed with water or honey solution and their longevity and female fecundity were compared between different food resources under caged conditions.

The hourly emergence pattern.

At first, the hourly emergence pattern of Japanese *A. aphidimyza* was investigated because no emergence curve has ever been elucidated until now. Hundreds of *A. aphidimyza* larvae were collected in early July 1998 from various localities in Fukuoka Prefecture, Japan, together with colonies of host aphids. These larvae and aphids were kept in rearing cylinders with moist soil, which keeps relative air humidity suitably high and provides pupation sites. These cylinders were incubated at 27°C under the conditions of natural photoperiod of Fukuoka Prefecture, about 14L:10D. The number of males and females there emerged from cocoons was recorded hourly for 4 successive dates after the first emergence date. Similarly, the hourly emergence pattern was also observed for 2 days for *A. aphidimyza* imported from The Netherlands. The hourly emergence patterns were compared between the Japanese and Dutch populations to detect the existence of any 'jet lag' for the latter.

RESULTS.

The failure of adult emergence from cocoons buried in vermiculite.

The selected bottle contained 1376 cocoons, from which 626 adults (45 %) successfully emerged. The contents of unsuccessful emergence were as follows: 504

(36.6 %) cocoons contained dead pupae, 165 (11.9 %) pupae died in the vermiculite layer after departing cocoons, 74 (5.4 %) adults died in the vermiculite layer on the way to the surface of the vermiculite layer, and 7 (0.5 %) adults died on the surface of the vermiculite layer. The emergence success from the cocoons that were buried in 10 mm depth of vermiculite layer was 66.2 %, while that from cocoons buried 150, 100, and 50 mm depth was extremely low (Fig. 1).

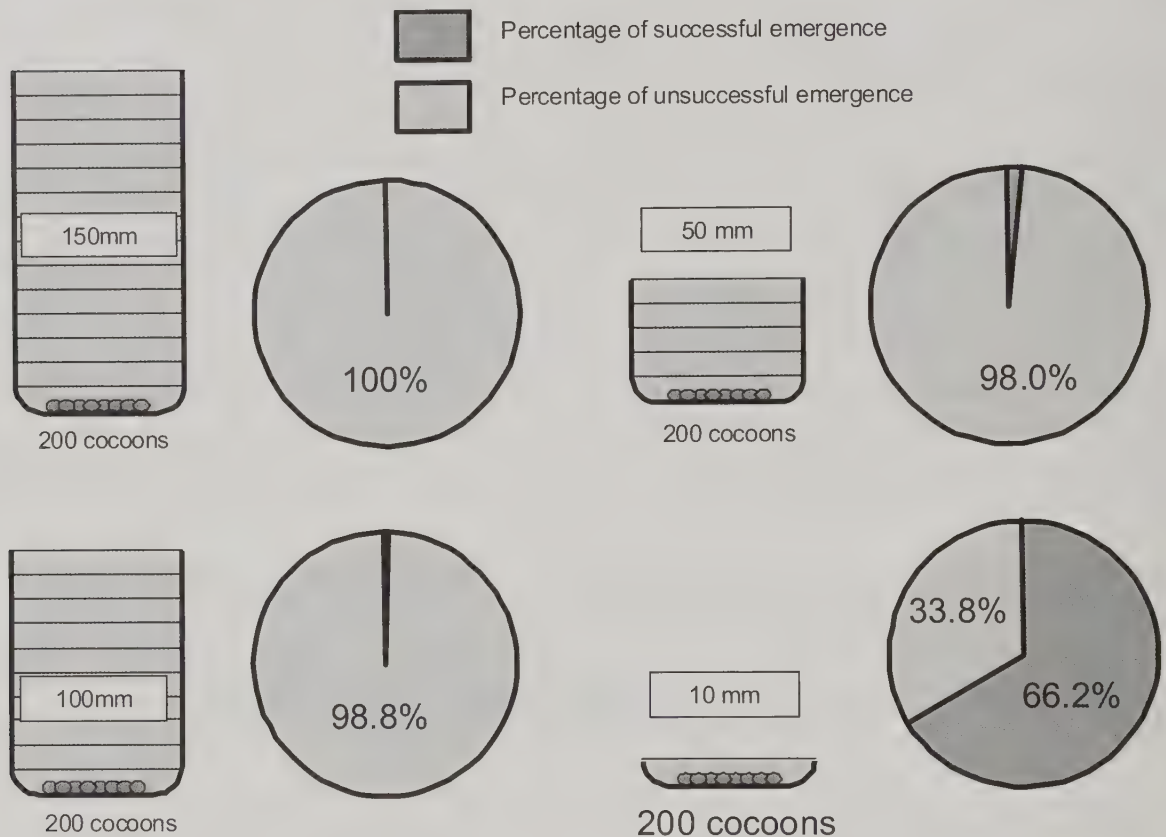


Fig. 1. Comparison in the percentages of successful and unsuccessful adult emergence from cocoons buried in different depths of vermiculite layer.

The importance of substrates for adults to hang on for successful mating.

Females that were transferred from the cylinder provided with fishing lines laid a total of 130 eggs on the strawberry leaves, while no eggs were laid by the females that were transferred from the cylinder without fishing lines (Table 1). This means that mating might not occur without substrates on which males and females can hang during mating.

The influence of nutrient on the longevity and fecundity of females.

There were no significant differences between water and honey solution in the longevity of males, while females fed with honey solution lived significantly longer than those with water alone (Table 2). Fecundity expressed as the number of eggs

laid during the adult lifespan was significantly greater in females fed with honey solution than in those with water alone (Table 2).

The hourly emergence pattern.

Adults of Japanese populations started to emerge before sunset, between 16h00 and 17h00, and the peak of emergence occurred sharply between 19h00 and 20h00 (Fig. 2). Similar hourly emergence curves were observed on the 2nd, 3rd, and 4th day. However, adults of Dutch populations started to emerge from 6:00 pm, and the peak of emergence occurred between 22h00 and 23h00 pm (Fig. 3). The emergence pattern in the 2nd day was similar to that in the first day, but the peak of emergence was not clear.

Table 1. Comparison of the number of eggs laid between females caged with males in a rearing cylinder provided with fishing lines as mating sites and those in a rearing cylinder without fishing lines.

Fishing lines to hang on	Provided	Not provided
No. of shoots on two strawberry plants	6	6
No. of leaves on two strawberry plants	18	18
No. of aphids on two strawberry plants	434	515
Total no. of eggs oviposited	130	0
No. shoots oviposited (%)	4 (66.7)	0 (0)
Mean number of eggs per shoot	21.7	0

Table 2. Comparison of mean (n=20).longevity and fecundity between *A. aphidimyza* fed with water and honey solution

Sex	Food	Mean longevity (days) ± s.d.	Mean fecundity (eggs) ± s.d.
Males	Water	4.11±2.88 ^{ab*}	--
	Honey	4.39±2.59 ^{ab}	--
Females	Water	2.11±0.94 ^a	5.05±7.17 ^x
	Honey	6.38±4.46 ^b	36.69±90 ^y

* Different letters in the same column indicate a significant difference: longevity was compared with Tukey-Kramer’s HSD test (p<0.05) and fecundity with t-test (p<0.05).

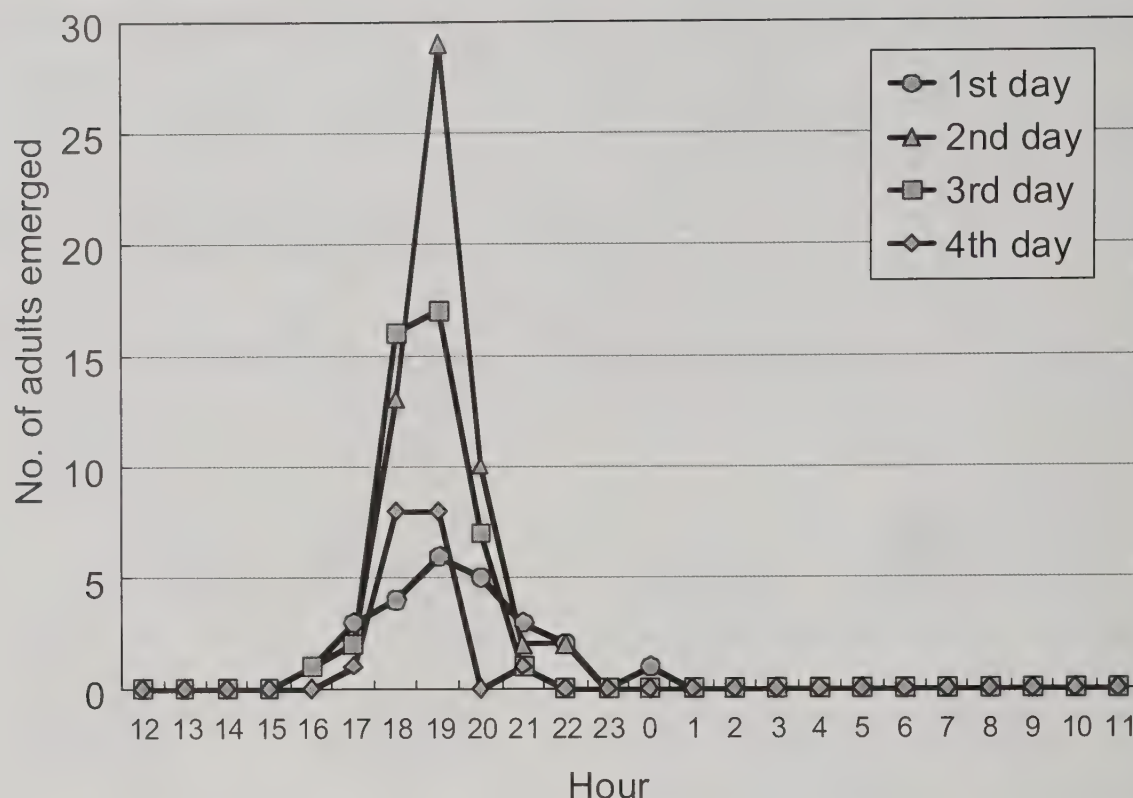


Fig. 2. Hourly emergence of *A. aphidimyza* collected in Japan (under natural photoperiod in Fukuoka City, Kyushu, Japan).

DISCUSSION.

The failure of adult emergence from cocoons buried in vermiculite.

In Japan, several releasing methods of imported cocoons have been suggested and employed by practitioners. For example, instructions say: “open the cap of bottles and leave them as they are in greenhouses, or divide cocoons and vermiculite substrates into several containers with 100 mm depth and leave them in the greenhouses”. However, our investigation revealed that cocoons should not be buried deeper than 50 mm to minimize the failure of adult emergence (Fig. 1). So, the instructions should be changed to “divide cocoons and vermiculite substrates into many shallow dishes with 10 mm depth and keep moistened for about three days until the final adult emerges from the cocoons”.

The importance of substrates for adults to hang on for successful mating.

Usually, premating swarming is required in many gall-inducing cecidomyiids (e.g., Yukawa *et al.* 1976), although a few species mate within a few minutes after emergence without swarming (e.g., Barnes 1946). Swarming occurs near to the host plant from which adults emerged in the case when pupation took place in the galls on the plant, or it occurs over the ground surface in the case when full-grown larvae drop to the ground for pupation (Yukawa & Rohfritsch 2005). In these cases, mating occurs on the host plant or on other substrates near the ground. However, *A.*

aphidimyza mates by hanging on abandoned spider-webs or other substrates in a face-to-face position. This seems to be a unique way of mating, but rather common to predacious cecidomyiid species and other nematoceran families in Diptera.

As demonstrated by our experiment, adults of *A. aphidimyza* cannot mate without suitable hanging substrates (Table 1). In greenhouses, suitable hanging substrates such as abandoned spider-webs do not always exist abundantly. Plant vines are usually too thick for them to hang on to. Therefore, provision of artificial substrates, such as very fine fishing lines, near release sites is essential to increase the successful mating rate in greenhouses.

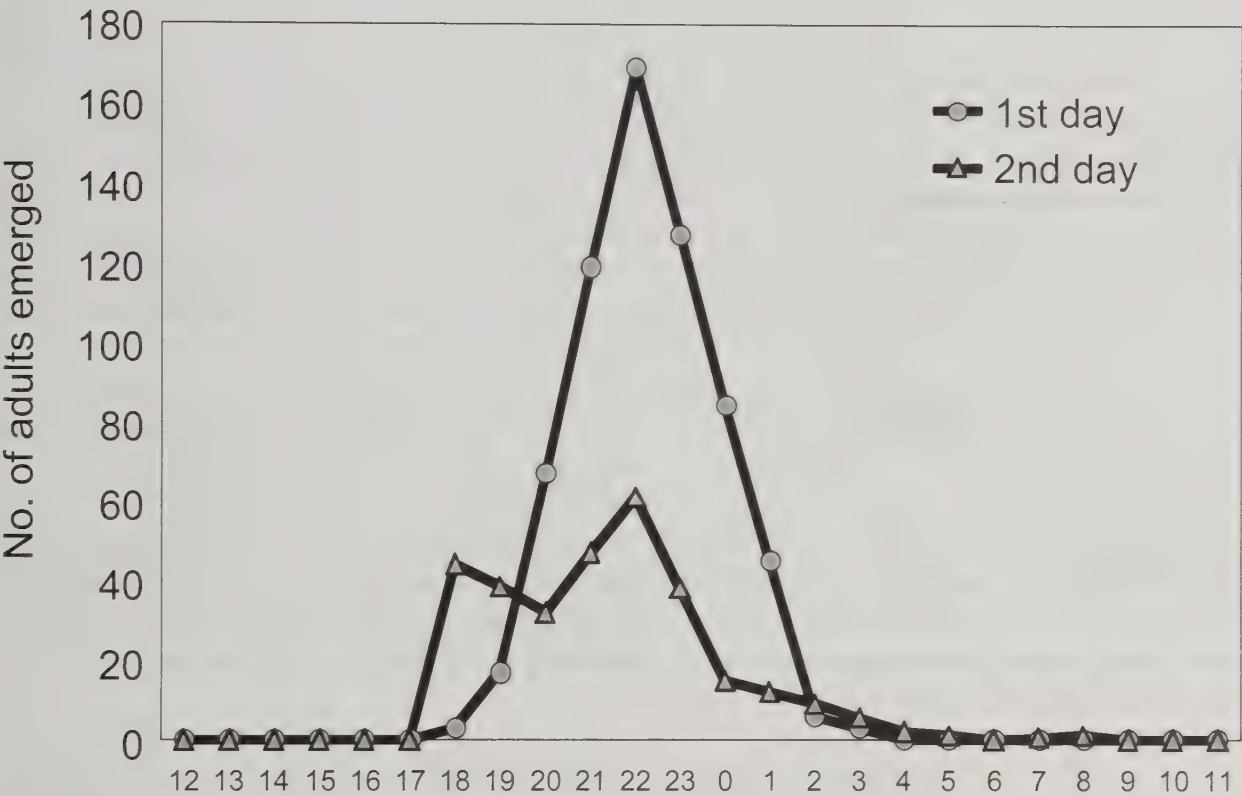


Fig. 3. Hourly emergence of *A. aphidimyza* imported from The Netherlands (under natural photoperiod in Kagoshima City, Kyushu, Japan).

The influence of nutrient on the longevity and fecundity of females.

Both sexes of *A. aphidimyza* are known to take aphid honeydew as an energy source (Harris 1973), but low fecundity has been cited as a disadvantage (Harris 1982; Chambers 1986). In previous studies, the fecundity of *A. aphidimyza* has been reported to vary from 6-38 to 156.6 eggs per female (Gilkeson 1987). It has been considered that such variations in fecundity might be related to variations in genetic makeup or larval nutrition (Kuo 1982; Havelka & Ruzicka 1984). Later, honeydew intake by females revealed that their life span was extended (Kuo 1985) and fecundity was increased (Sell and Kuo-Sell 1987).

Our investigation also demonstrated that the intake of honey solution extended female longevity and increased fecundity (Table 2). If artificial food resources, such as honey solution, are provided from place to place in greenhouses, *A. aphidimyza* females would have more chance to take nutrients than in the situation when only natural honeydew is available. In particular, artificial food resources would become more effective when aphid density is relatively low, so that honeydew production is limited. This is one of the important points in the practical use of *A. aphidimyza*, because it is desirable to release *A. aphidimyza* before the aphid population increases to a high level.

The hourly emergence pattern.

The emergence of many gall-inducing cecidomyiid species extends over several hours, and attains its maximum in numbers at a time that is constant for any one species (e.g., Barnes 1930; Yukawa *et al.* 1976), although some exhibit bimodal emergence pattern as has been noted for some nocturnal species of *Asphondylia* (Diptera: Cecidomyiidae) (e.g., Yukawa & Miyamoto 1979; Yukawa & Ohsaki 1988). *A. aphidimyza* adults have been known to emerge during the daytime (Harris 1973) but no detailed hourly emergence curve has ever been elucidated until now. In this study, individuals of the Japanese population started to emerge a few hours before sunset, and the emergence reached a peak after sunset (Fig. 2). Similar patterns were observed over several successive days. This species is nocturnal and is generally active between sunset and sunrise: they do not fly actively in the daytime and remain immobile in shaded parts of the plant (Harris 1973; Makkula & Tittanen 1985). Therefore, the hourly emergence pattern, which is not bimodal, and daily activities are considered to be common to both Japanese and European populations of *A. aphidimyza*. Nevertheless, the peak emergence of *A. aphidimyza* imported to Japan from The Netherlands occurred between 22h00 and 23h00 (Fig. 3), a delay of about three hours compared with the Japanese population. This indicates some influence of jet lag on the time of emergence.

In Japan, the temperature in greenhouses markedly drops to nearly 5°C for strawberry and 14-16°C for eggplant and sweet pepper, particularly in the winter season between midnight and sunrise. When imported *A. aphidimyza* adults emerge at about midnight, they cannot behave actively under the relatively cold conditions, resulting in the failure of mating and oviposition. Therefore, rearing conditions should be changed in the place of origin for exporting cocoons to countries with a time difference of more than a few hours. Otherwise, effective measures to relieve jet lag should be developed in importing countries.

General concern for contamination of native Japanese populations with European ones.

In addition to our proposals for improvement in the practical use of *A. aphidimyza*, special attention should be paid to the possible genetic and ecological contamination of native Japanese populations with European ones. If *A. aphidimyza* cocoons are continuously imported to Japan from The Netherlands, it will be highly probable that European *A. aphidimyza* will escape from greenhouses and will contaminate the Japanese population.

Previously, the identification of *A. aphidimyza* was based only on morphological characters, and there are no clear diagnostic morphological differences between Japanese and European specimens (Yukawa *et al.* 1998). Therefore, Shirota *et al.* (1999) tried to find substantial genetic variability in the mitochondrial cytochrome oxidase subunit I (COI) gene of *A. aphidimyza*. According to their molecular data, there were clear genetic differences between Japanese and Dutch populations. The number of substitutions of the Dutch population compared with the Japanese population was 31 to 33 of 314 bp. Thus, the partial COI gene of *A. aphidimyza* can be useful as it contains genetic markers to monitor the introgression of European traits into Japanese strains. Our recent preliminary experiments of cross mating between Japanese and Dutch populations indicated that about half of the eggs could not give rise to F₁ progeny. This may mean that the two populations have started to diversify into different species, and if so, genetic contamination would hardly occur. However, ecological interference still exists between them.

To avoid contamination, *A. aphidimyza* should be mass-produced in Japan using Japanese populations, but mass-rearing methods of the species have not yet been established in Japan, and the Asian market is too small at the moment to support production of cocoons on a commercial scale. Therefore, it is desirable that companies should have a keen awareness of the problem and begin to mass-produce individuals originating in target countries of exportation.

Possible effects of global warming on aphid populations.

Aphids have a great potential to increase the number of annual generations under global warming because of their low developmental zero point and low amount of effective cumulative temperature required for one generation (Yamamura and Kiritani 1998). Therefore, more attention has to be paid to aphid control in greenhouses as a model case of global warming.

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SESSION 3

EXPLORING BIOLOGICAL CONTROL TO MANAGE NEW OR POTENTIAL INVASIVE ALIEN PESTS

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Excepting biopesticide sprays, biological control has been used almost exclusively to manage exotic arthropod pests which are already well-established and causing measurable economic damage. However, pest invasions are becoming increasingly well understood, so that the likely local impacts of a new arthropod pest and its candidate biocontrol agents can often be predicted from their effects elsewhere coupled with eco-climate matching. This means that biological control is becoming an increasingly feasible option for managing alien pests in the early stages of their invasions. Nevertheless, the use of biological control against low-density invading pest populations presents some particular technical challenges, such as: (1) selecting appropriate agents given a paucity of local host data; (2) establishing biocontrol agents in low-density host populations; and (3) robustly measuring biocontrol impacts in the absence of base-line equilibrium pest data. This session uses a range of case studies, from temperate and tropical, continental and island ecosystems, to explore the potential for biological control to manage new or potential invasions of arthropod pests. The primary aims are to demonstrate the feasibility of this approach, identify particular challenges, and show how carefully planned experiments, population models and ecological theory can help maximise the chance of success. It is hoped that these examples will provide the inspiration, insight and experience needed to make biological control a useful tool for managing invasive alien pests before they can cause significant local damage.

CAN MATRIX MODELS GUIDE THE SELECTION OF PARASITOIDS FOR BIOLOGICAL CONTROL INTRODUCTIONS?: LBAM IN CALIFORNIA AS A CASE STUDY

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ABSTRACT.

The light brown apple moth (LBAM, *Epiphyas postvittana*) invaded California from S.E. Australia in 2007 and has become established in two coastal regions around San Francisco and Santa Cruz. As LBAM is not considered a key pest in Australian crops, due to the activity of its natural enemies, one of the management options for this new invader in California is classical biological control. From a series of studies in Australia much is known of the life history and population ecology of LBAM, and as is typical for Lepidoptera, LBAM supports a species rich parasitoid community. In an effort to maximize the success of this project and to guide the selection of parasitoid species for host range testing and potential introduction into California, we make use of a stage-structured matrix model to analyze the life cycle of LBAM for vulnerabilities to parasitism. This approach has been used in biological weed control to guide classical introductions, but has been neglected as a tool for the selection of parasitoids to use against new invasive insect pests. Prospective analysis of a matrix model for LBAM suggests that the mid larval and pupal stages are the most vulnerable stages of the life cycle, and thus parasitoids impacting these life stages have been prioritized for host range testing and potential introduction. Matrix models are likely to serve as a valuable tool to guide the selection of parasitoids for other invasive insect pests that support a species rich parasitoid community in their region of origin.

INTRODUCTION.

California agriculture is a \$32 billion industry and one of the state's leading sources of revenue and employment. In March 2007, the confirmation of the light brown apple moth (LBAM) *Epiphyas postvittana* (Walker) (Lep.: Tortricidae) in California posed a new threat to the California agricultural industry with the potential to cause millions of dollars in damage to agricultural, specialty and ornamental crops (Varela *et al.* 2008). LBAM originates from SE Australia, has been recorded from more than 250 plant species including fruit, ornamentals, vegetables and weeds, and has become a well-known pest in New Zealand where it is a pest of fruit crops such as apples and grapes (Wearing *et al.* 1991). In California, LBAM has subsequently been found in 12 counties in the San Francisco Bay Area and the Monterey Bay Area. The Animal Plant Health Inspection Service arm of the United States Department of Agriculture issued a Federal Domestic Quarantine order in May 2007, with restrictions on interstate shipment of plant material, and the California Department of Food and Agriculture (CDFA) has issued a State Interior Quarantine order restricting intrastate shipment of plant material from counties where light brown apple moth is known to occur.

The current response to this new invasion is to eradicate LBAM from California and a variety of approaches that include sterile insect technology, pheromone technology, augmentative releases of *Trichogramma* egg parasitoids, and foliar sprays of Bt and/or spinosad are being considered (CDFA 2008). As a more long term management option for LBAM, classical biological control is also being considered, should eradication not be feasible or possible. A recent review of the parasitoids reared from LBAM collected from agricultural crops in Australia by Paull & Austin (2006) provides an excellent starting point for consideration of which species might be suitable for introduction into California for this purpose. Two of the most important criteria for selection of natural enemies for introduction in biological control programs are host specificity and potential impact (Sheppard *et al.* 2003; Goolsby *et al.* 2005; McClay & Balciunus 2005; Mills 2005). While much attention has been focused on host specificity, there has been far less consideration of potential impact for introductions of parasitoids for biological control of insect pests, and it is this component of the selection process that I will focus on here in the context of parasitoid introductions from Australia for biological control of LBAM in California.

There are a number of different ways in which the potential impact of an exotic parasitoid of an invasive insect pest might be evaluated, and although used more extensively in biological weed control, there are very few specific examples from the biological control of insect pests. Approaches that have been used include estimation of rates of parasitism in the region of origin prior to collection (Mills 2005), estimation of rates of parasitism in cages in quarantine (Goolsby *et al.* 2005), tactical modeling as pioneered by Godfray & Waage (1991), and the use of matrix models to identify the most vulnerable stages in the life cycle of the pest (Mills 2005). One of the most promising of these approaches that has been used more broadly and effectively in biological weed control (McEvoy & Coombs 1999; Raghu *et al.* 2006), is the evaluation of vulnerabilities in the life cycle and it is this approach that will be explored further in the context of selecting Australian parasitoids that are most likely to impact populations of LBAM in California.

PARAMETERIZING A STAGE-STRUCTURED MATRIX MODEL FOR LBAM.

Benton & Grant (1999) provide a valuable introduction to the application of matrix models in applied ecology, including biological control, and Caswell (2001) provides a detailed guide to their use. Life cycle stages included in the matrix model are egg, 1st instar larva (L_1), 2nd-5th instar larva (L_{2-5}), 6th instar larva (L_6) and pupa to match life table data provided by Danthanarayana (1983), and prereproductive (Ad_{pr}) and reproductive (Ad_r) adult females to effectively capture the onset of reproduction in this species (Fig. 1). The projection matrix developed for LBAM (Table 1) consists of a set of upper diagonal elements (P_i) representing the probability of stasis in which an individual survives and remains within the same life stage, and a set of lower diagonal elements (G_i) representing the probability of transition in which an individual survives and moves on to the next life stage. These probabilities are dependent upon the survival (σ_i) and development (γ_i) rates of each life stage (i) in relation to the time step of the projection (1 day), such that $P_i = \sigma_i(1 - \gamma_i)$ and $G_i = \sigma_i\gamma_i$.

The model was parameterized for California populations of LBAM using data available from studies carried out in Australia. Daily survival rate for each juvenile life

stage was estimated as the mean survivorship from the 16 life tables in Danthanarayana (1983) from the La Trobe University site near Melbourne in Australia excluding mortality due to parasitism, as these parasitoids are not present in

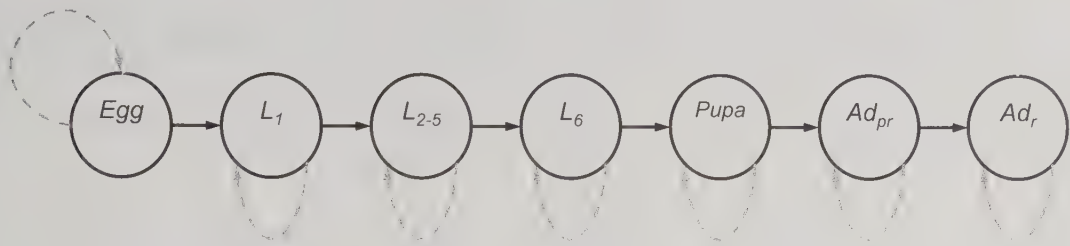


Fig. 1. The life cycle diagram for LBAM showing the successive life stages with connectors representing the probability of stasis (P_i , dashed gray arrows), the probability of transition to the next life stage (G_i , solid black arrows), and recruitment from the reproductive adult stage to the egg stage (F , dotted gray arrow).

California. Mean survivorship was converted to daily survival rate, assuming a constant rate of mortality through the life stage, using the expression $\sigma_i = s^{1/d}$ where s is mean life stage survivorship and d is mean life stage duration. Life stage durations were taken from data provided by Danthanarayana *et al.* (1995) for a constant temperature of 19.9°C, with the duration of the complete larval stage broken down into the three larval instar ranges used in the model from personal observations of the duration of 1st and 6th instar larvae.

Table 1. The projection matrix for LBAM with diagonal elements representing the probability of survival and remaining in the same life stage (upper element) and the probability of survival and transitioning to the next life stage (lower element), and the upper right hand element representing the daily per capita realized production of female eggs. Values derived from Danthanarayana (1975, 1983), Danthanarayana *et al.* (1995) and Robison *et al.* (1998) as indicated in the text.

	Egg	L ₁	L ₂₋₅	L ₆	Pupa	Ad _{pr}	Ad _r
Egg	0.815	–	–	–	–	–	12.125
L ₁	0.118	0.718	–	–	–	–	–
L ₂₋₅	–	0.144	0.910	–	–	–	–
L ₆	–	–	0.066	0.813	–	–	–
Pupa	–	–	–	0.163	0.878	–	–
Ad _{pr}	–	–	–	–	0.103	0.625	–
Ad _r	–	–	–	–	–	0.313	0.844

The mean longevity of the prereproductive and reproductive periods of adult female life and the progeny sex ratio were taken from Danthanarayana (1975), while

data on female fecundity in the laboratory at 19.9°C was obtained from Danthanarayana *et al.* (1995). The daily survival rate of adult females was estimated, after Caswell (2001) as $1 - (1/\text{mean longevity})$, and their daily realized fecundity was estimated as $F = \text{sex ratio} * (\text{lab fecundity/reproductive period}) * \text{proportion realized}$. While loss of potential fecundity is often not included in life table data, such as that from Danthanarayana (1983) for LBAM, the fecundity realized under field conditions is often only a proportion of that measured under laboratory conditions (Price *et al.* 1990). The proportion of the laboratory fecundity realized was estimated from data provided by Robison *et al.* (1998) for another archipine tortricid *Choristoneura fumiferana*.

PROSPECTIVE ANALYSIS OF THE LBAM MATRIX MODEL: LIFE CYCLE VULNERABILITIES.

To be able to ask the question: ‘If parasitism reduced the survival rate of a particular stage in the life cycle of LBAM what effect would it have on population growth rate?’ we use prospective analyses of the LBAM matrix model. One approach is to evaluate the impact of a very small proportional change in value for each matrix element, or their component vital rates, on population growth rate (λ). Referred to as the elasticity of λ to the matrix element or vital rate, elasticity analysis has been widely used. Benton & Grant (1999) point out that considerable caution must be used in extrapolating the results of elasticity analysis to the impact of larger changes in vital rates on population growth rates due to inherent nonlinearities and the unequal durations of life stages represented in stage-structured models. Nonetheless, de Kroon *et al.* (2000) and Caswell (2001) indicate that in practice elasticity analysis does a good job of predicting the effects of large perturbations in vital rates. Thus as a first step toward prospective analysis of the likely impact of

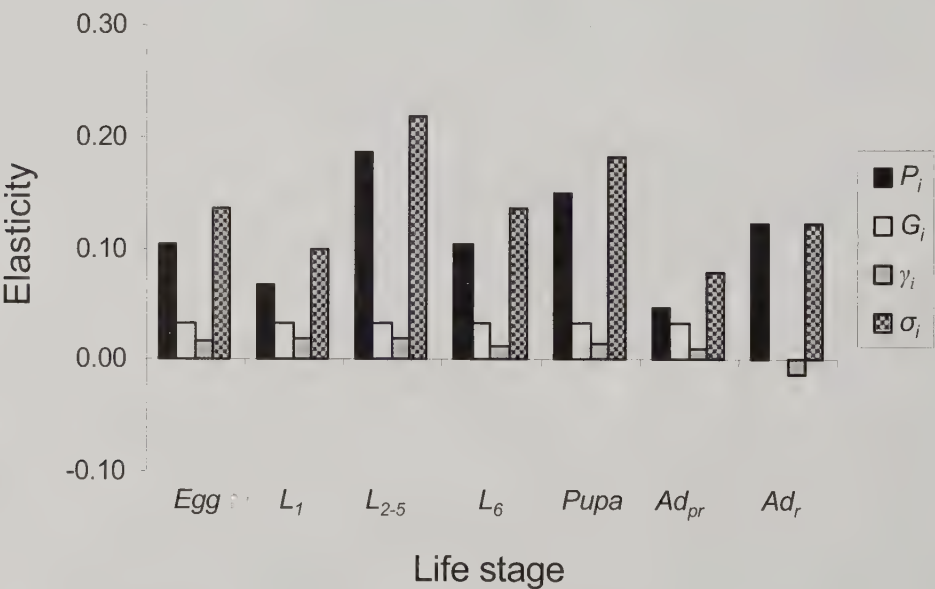


Fig. 2. Prospective analysis to show the elasticity of λ to the stasis (P_i) and transition (G_i) elements of the matrix model for LBAM, and their subcomponent vital rates of development rate (γ_i) and survival rate (σ_i) for each life stage.

adding parasitism at different life cycle stages to the population growth rate of LBAM we use elasticity analysis, both for the stasis and transition elements of the matrix, and for their component vital rates (Fig. 2). In addition, the elasticity of λ to daily realized fecundity (F) is 0.032 (not shown in Fig. 2). The larger the elasticity, the greater its impact on the population growth rate of LBAM. Thus it can be seen for all life stages that the probability of stasis has a greater influence than the probability of transition, and that this is due to the greater influence of the component survival rates compared to development rates. Looking for vulnerabilities (the largest elasticities) in the life cycle of LBAM, we can also clearly see that changes in survival rate of the mid larval stage (L_{2-5}) and the pupal stage have the greatest impact on population growth rate.

As a second step toward prospective analysis of the likely impact of adding parasitism at different life cycle stages, we can also examine the extent to which survival rate would need to be reduced at a particular life cycle stage to suppress population growth (i.e., to reduce λ to 1). This approach has been used previously by Lin & Ives (2003) and Mills (2005) to further verify the life cycle vulnerabilities identified through elasticity analysis. The daily survival rate from added parasitism (σ_p) can be incorporated into the matrix model elements as $\sigma_{p_i} * P_i$ and $\sigma_{p_i} * G_i$ where for each life stage i , p_i is the level of parasitism contributed by an introduced parasitoid at that life stage and $\sigma_{p_i} = (1 - p_i)^{1/d_i}$. By incorporating parasitism into individual life stages (egg through pupa) while keeping other life stages free from parasitism, we can determine the rate of parasitism required to generate zero population growth for LBAM. These rates of parasitism thus provide a further indication of the relative vulnerability of the different life cycle stages of LBAM to the impact of introduced parasitoids acting at different stages in the life cycle.

Using this second approach to prospective analysis of the LBAM matrix model, we see that there is a very good match between the rates of parasitism required for zero population growth at each life stage and the corresponding elasticities of λ to survival rates (Fig. 3). Those life stages with the greatest elasticity of λ to survival rate require the lowest rates of parasitism to suppress population growth and again highlight the mid larval (L_{2-5}) and pupal stages as being most vulnerable. The negative relationship is perfectly linear giving us further confidence that we have correctly identified these two life stages as most vulnerable to introduced parasitoids in the life cycle of LBAM.

CONCLUSIONS: A STRATEGY TO PRIORITIZE PARASITOID INTRODUCTIONS.

In a recent review, Paull & Austin (2006) reviewed what is known of the parasitoids reared from LBAM collected from a variety of agricultural crops in Australia. A schematic form of this information (Fig. 4) summarizes the species present in the parasitoid community, together with basic details of the parasitoid guilds to which they belong (*sensu* Mills 1994). With such a wide range of parasitoids to select from this clearly poses a considerable problem with regard to host specificity screening. However, using the prospective analyses of the matrix model for LBAM as a guide to likely impact, we can see from the schematic summary (Fig. 4) that there is only a single parasitoid species, *Dolichogenidea tasmanica* (Cameron) (Hym.: Braconidae), that can influence the survival rate of the mid larval stage (L_{2-5}), although there are

still 7 parasitoid species that could influence the survival rate of the pupal stage (those attacking the late larval stage as well as those attacking the pupal stage).

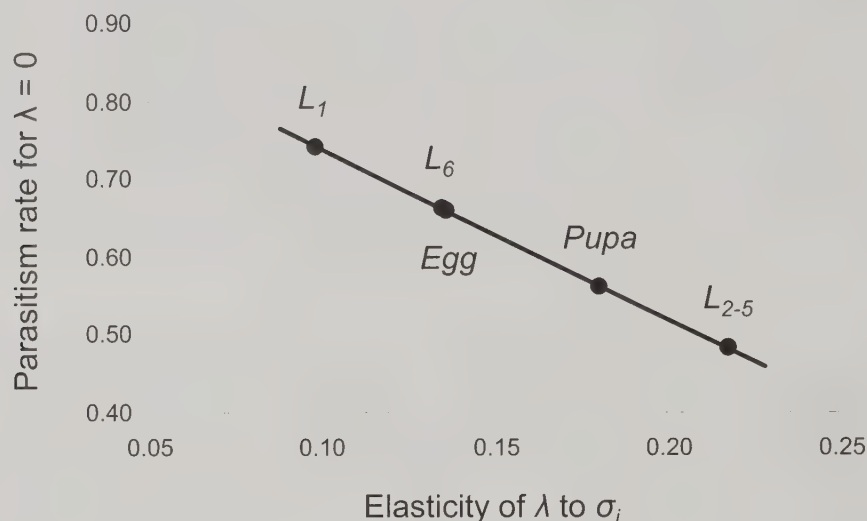


Fig. 3. A comparison of two approaches to prospective analysis of the LBAM matrix model: the relationship between rate of parasitism required to suppress population growth rate and the elasticity of population growth rate to survival rate for each life stage.

Among these later two parasitoid guilds, however, it is already known from New Zealand that the tachinids and the ichneumonid pupal parasitoids are polyphagous species (Thomas 1989; Munro 1998), leaving *Exochus* sp. (Hym.: Ichneumonidae) and the two *Brachymeria* species *B. teuta* (Walker) (Hym.: Chalcididae) and *B. phya* (Walker) (Hym.: Chalcididae) as potential candidates. Thus, as a result of using the stage-structured matrix model we have been able to prioritize those parasitoids that are most likely to have a greater impact on the population growth rate of LBAM for host range testing. We are currently testing the host range of *D. tasmanica* and will soon be carrying out similar tests with *Brachymeria teuta* as these are two of the most common parasitoids of LBAM in Australia, while both *Exochus* sp. and *B. phya* appear to be less common associates of LBAM.

Plant-feeding insects differ considerably in the species richness of their parasitoid communities, which is influenced by host abundance, taxonomy, and feeding niche (Hawkins 1994). In the context of classical biological control, when the richness of the parasitoid community is high, as is the case for many lepidopteran, symphytan and leafmining hosts, it is of particular importance to be able to prioritize the host range testing of potential candidates for introduction, not only to optimize the timeline of the project, but also to reduce the cost of the background research required. Matrix models to estimate the likely impact of parasitoids at different life cycle stages offer a very valuable tool for the optimization of such decision-making in classical biological control.

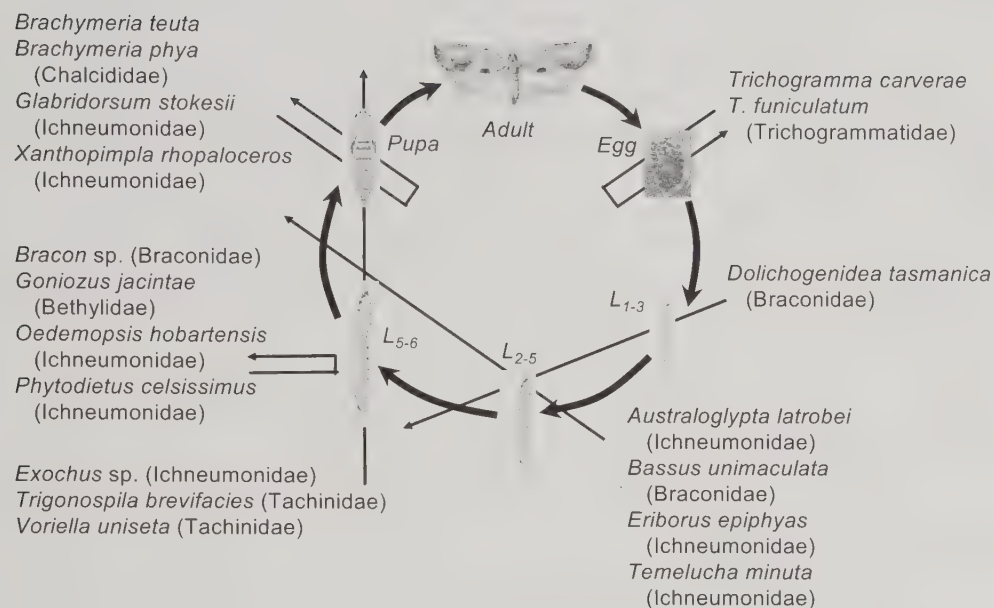


Fig. 4. A schematic representation of the life cycle of LBAM and the more frequently associates members of the parasitoid community, indicating timing of host attack (origin of the arrows) and host death (end of the arrows) and distinguishing between endoparasitoids (arrows passing through the circle) and ectoparasitoids (arrows remaining outside of the circle).

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EXPLORING BIOLOGICAL CONTROL TO MANAGE INVADING POPULATIONS OF CLOVER ROOT WEEVIL

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ABSTRACT.

The clover root weevil, *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae), has been well-established in the North Island of New Zealand for over a decade, but has only recently been found in parts of the South Island. A parthenogenetic strain of the parasitoid wasp *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) was introduced into the North Island in January 2006 and is promising to achieve effective biological control. This system presents a rare opportunity to trial classical biocontrol as a strategy for slowing the establishment and spread of early invasive clover root weevil populations in the South Island. Mass-emergence devices are being investigated for releasing the parasitoid into low-density weevil populations at the leading edge of the invasion, and sampling is being undertaken to assess whether the parasitoid population can keep pace with the spread of the host. In addition, population models are being used to explore the circumstances under which natural enemies may prevent or slow the spread of invading insect populations, as has been previously demonstrated with weeds.

ENGINEERING AN INVASION IN TROPICAL ISLANDS: CLASSICAL BIOLOGICAL CONTROL OF THE GLASSY-WINGED SHARPSHOOTER, *HOMALODISCA VITRIPENNIS*, BY THE EGG PARASITOID *GONATOCERUS ASHMEADI* IN FRENCH POLYNESIA

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ABSTRACT.

Tropical islands are paradises for potential invaders. They offer ideal conditions for pest proliferation: mild climate, numerous host-plants, and few competitors and natural enemies. The invasion of the glassy-winged sharpshooter, *Homalodisca vitripennis* Germar (= *H. coagulata* Say) (Hemiptera: Cicadellidae) in French Polynesia is a typical example. *Homalodisca vitripennis* was first recorded in Tahiti in 1999. It reproduced and spread very rapidly and a few years later was found in 10 islands of French Polynesia in three different archipelagos. It became an important pest threatening agriculture and native biodiversity, and created intolerable social and recreational problems. Further, massive uncontrolled populations on Tahiti presented an elevated invasion threat to other South Pacific nations. To minimize these problems, a classical biological control program against *H. vitripennis* was conducted using the host specific egg parasitoid *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae). After risk assessment studies indicated an acceptably low level of risk to non-target species, parasitoids were released in Tahiti in 2005. Within a few months, the parasitoid colonized all Tahiti and all other infested islands in French Polynesia. The impact of *G. ashmeadi* on *H. vitripennis* was extremely rapid and catastrophic in all infested islands. Arrival of *G. ashmeadi* slashed *H. vitripennis* densities by more than 95%. Pest populations were maintained at very low densities until now in spite of seasonal fluctuations. French Polynesia was a paradise for *H. vitripennis* and became a paradise for *G. ashmeadi*.

INTRODUCTION.

Invasive species are exotic species introduced intentionally or by accident, in a new environment where they proliferate in detriment of indigenous species. Invasive species are the second cause in loss of biodiversity after habitat destruction in continents, and the first cause in islands (Wilcove *et al.* 1998). The many, small, isolated islands of French Polynesia are extremely susceptible to invasions, because of the low number of species, the high endemism and because of intensive trade with surrounding countries (Perrings *et al.* 2002). In addition, tropical islands are especially susceptible to invasion because they have climatic conditions favorable to the reproduction and proliferation of numerous species.

French Polynesia has a long history of biological invasions: both intentional (e.g. the giant African land snail, *Achatina fulica* Bowdich (Mollusc)) and accidental (e.g. the little fire ant *Wasmmania auropunctata* Roger (Formicidae, Myrmicinae). Classical biological control was the answer to several invasions in the past, but the poor track record in biological control safety and program efficacy led to dramatic consequences in several cases. The most egregious example of a “biological control disaster” in French Polynesia was the unintentional extirpation of native *Partula* snails on many islands by the predatory snail, *Euglandina rosea*, that was released in 1977 for the biological control of giant African land snail, *Achatina fulica* (Murray *et al.* 1988). Presently, classical biological control of invasive species in French Polynesia is considered with skepticism and is considered only following: 1) demonstration that there is no other feasible solution, 2) studies assessing the risk of the biocontrol agent for the native fauna, and 3) an impact study of the target species, and if possible, non-target species. A case study was the classical biological control program against the glassy-winged sharpshooter *Homalodisca vitripennis* Germar (= *H. coagulata* Say) (Hemiptera: Cicadellidae).

The glassy-winged sharpshooter is native to the southeastern U.S.A. and northeastern Mexico (Triapitsyn & Phillips 2000). It is a polyphagous sharpshooter that can feed and develop on more than 150 plant species in 34 plant families (Hoddle *et al.* 2003). It is a major pest of agricultural, ornamental and native plants, because of its ability to vector the plant bacteria *Xylella fastidiosa* (Wells *et al.* 1987), which causes a variety of lethal scorch-like diseases in susceptible hosts (Hopkins & Purcell 2002). It invaded California at the end of the 80's and is now spreading to Pacific islands. *Homalodisca vitripennis* was first recorded in Tahiti in 1999. It likely invaded as eggs on ornamental plants imported from California. It reproduced and spread very rapidly in French Polynesia reaching very high densities in Tahiti and nearby Moorea and invaded eight other islands located in three different archipelagos of French Polynesia by 2005 (Fig. 1). The invasion dynamics of *H. vitripennis* in French Polynesia is detailed in Petit *et al.* (2008a), who demonstrated the role of human-mediated transport in the spread of this pest. The bacteria *X. fastidiosa* was not recorded in French Polynesia but the extremely high densities of *H. vitripennis* in Tahiti and Moorea threatened agriculture and native biodiversity and created social nuisance. These problems was mainly due to prolific feeding of *H. vitripennis* which caused impaired growth of plants, created aesthetic problems due to excreta. Recreational activities under trees were impossible due to excreta dripping from trees, and large numbers of insects invaded houses at night due to attraction to light. Further, massive uncontrolled populations on Tahiti presented an elevated invasion threat to other Pacific islands and trading partners (Grandgirard *et al.* 2006). This threat was realized when *H. vitripennis* was discovered on Easter Island in 2005 and the Cook Islands (Rarotonga) in 2007 (Petit *et al.* 2008a). The major factors responsible for successful and rapid invasion of *H. vitripennis* in French Polynesia are: permissive environmental conditions (mild climate and abundant year-round feeding and oviposition substrates) leading to a year-round reproduction of this pest with 7-8 overlapping generations a year; absence of host specific natural enemies; death of generalist predators that attack nymphal and adult stages, and limited competition for resources (Grandgirard *et al.* 2006, Suttle & Hoddle 2005).



Fig. 1. *Pandanus* sp. Infested by *Homalodisca vitripennis* in Tahiti (2005, before the release of the parasitoid *Gonatocerus ashmeadi*) (Photo: J. Petit).

Due to the wide distribution and high abundance of *H. vitripennis* in French Polynesia and the absence of specific natural enemies, classical biological control was considered as the most appropriate solution to combat this pest and minimize its associated problems. A classical biological control program was conducted between 2004 and 2007 using the parasitoid *Gonatocerus ashmeadi* Girault (Hymenoptera: Cicadellidae). This parasitoid, native to southeastern U.S.A. and northeastern Mexico (Triapitsyn *et al.* 1998), was chosen because of its high specificity for eggs of Proconiini sharpshooters and promising control capacities exhibited in California and Hawaii (Triapitsyn *et al.* 1998; Logarzo *et al.* 2003; Bautista *et al.* 2005; Pilkington *et al.* 2005). The present paper gives an overview of this program.

RISK ASSESSMENT TO NATIVE CICADELLID FAUNA. (from Grandgirard *et al.* 2007)

A priori risk assessment studies were performed in the Society Islands before the introduction of the parasitoid in French Polynesia. Initial releases of *G. ashmeadi* were planned for Tahiti (Society Islands) because Tahiti had the highest pest densities. Also, Tahiti is the main island of French Polynesia and is the major hub for domestic and international air and sea transport. It thus represented the most likely source of *H. vitripennis* spreading to new areas (Petit *et al.* 2008a). An inventory of the Cicadellidae of the Society Island archipelago was undertaken and the risk of attack by *G. ashmeadi* was assessed using four criteria: 1) phylogenetic relationships between non-target cicadellids and known hosts for *G. ashmeadi*, 2) morphology, 3) egg laying biology, and 4) ecology. A risk decision tree was used to test criteria 3 and 4. Important knowledge on the host range of *G. ashmeadi*, and the behavior, ecology and taxonomy of native cicadellids from southern California facilitated the

development of this dichotomous risk decision tree. This tree is a useful tool that could be used in other countries (Fig. 2).

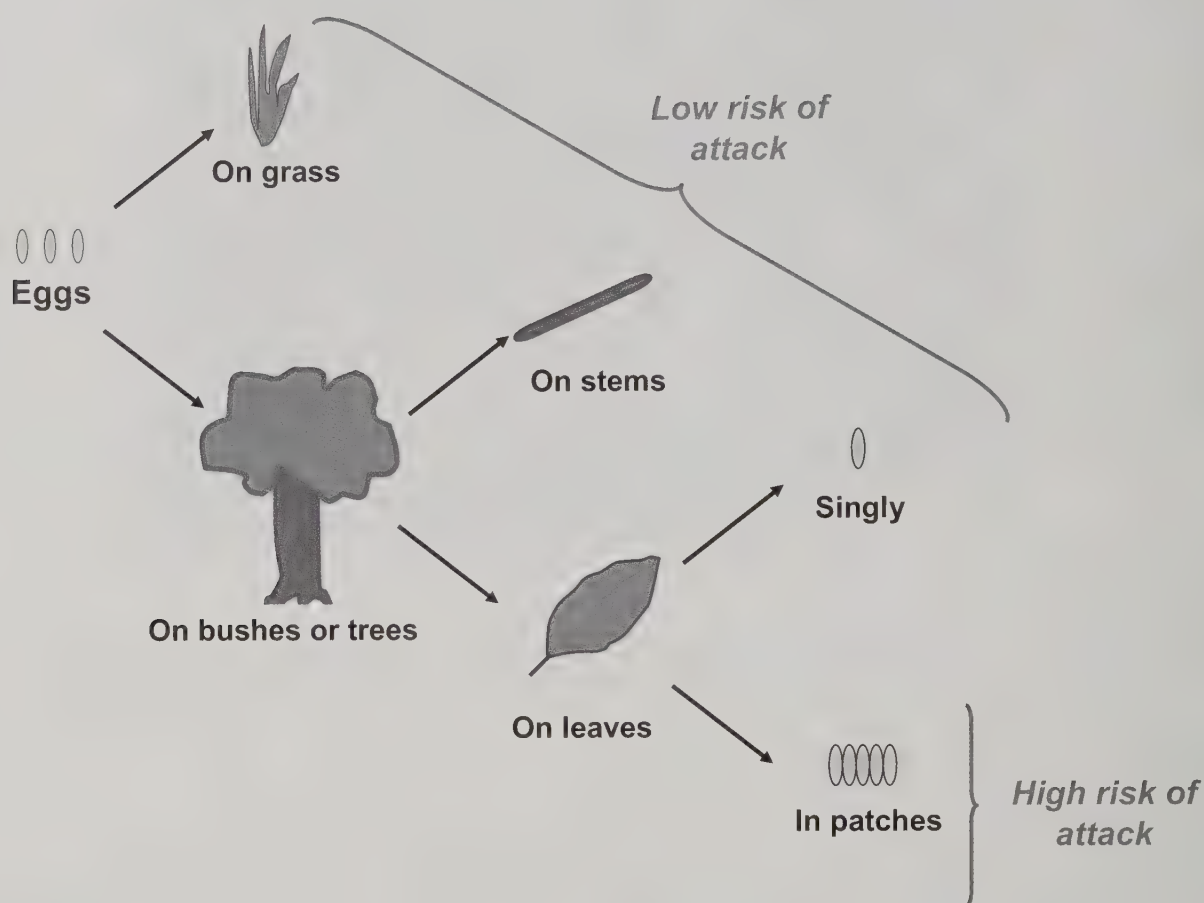


Fig. 2. The dichotomous risk-decision tree used to determine the risk posed by *Gonatocerus ashmeadi* releases on cicadellids native to French Polynesia. This tree enabled rapid assessment of the risk of attack by *G. ashmeadi* on eggs of native cicadellids (from Grandgirard *et al.* 2006).

All native cicadellid species found (14 species at the time of release decision) were considered to be at low risk of attack because they differed greatly from all known hosts for *G. ashmeadi*: (1) none are in the tribe Proconiini, 2) all were very small and, when determined, 3) lay tiny single eggs, which 4) are deposited on the leaves of trees. Hence, the French Polynesian government authorized the release of *G. ashmeadi* in Tahiti in April 2005. The risk assessment study continued *a posteriori* (after release in Tahiti) in Society Islands and was extended to other archipelagos (Marquesas and Australs) before planned releases in these archipelagos (2006). All native cicadellids found (> 25 species) were shown to have a very low risk of attack by *G. ashmeadi*.

RELEASE AND DISPERSAL OF BIOCONTROL AGENT. (from Petit *et al.*, 2008b, 2008c)

Gonatocerus ashmeadi was first released on Tahiti in May 2005. A total of 13,786 parasitoids were released at different 27 sites around Tahiti between May and

October 2005 (Grandgirard *et al.* 2007; Grandgirard *et al.* 2008a). Short and long-distance dispersal of *G. ashmeadi* were studied. This study was an opportunity to identify invasion pathways of its host and to study the invasion process of an invasive species (i.e. the deliberate introduction of *G. ashmeadi*).

Short-distance dispersal.

The parasitoid spread very rapidly in Tahiti and had colonized the entire island by October 2005. Results demonstrated that *G. ashmeadi* exhibits an exponential dispersal capacity. Survey results across different altitudes revealed an effect of vegetative diversity and host density on the mobility and establishment of *G. ashmeadi*, suggesting that many small releases would be needed for establishment when host density is high and larger and fewer releases when host density is low. Results also strongly suggested *G. ashmeadi* should be able to suppress rapidly *H. vitripennis* in every new area that this pest establishes high density populations if the climate is permissive enough to allow year round reproduction by the pest (Hoddle, 2006).

Long-distance dispersal.

All islands of French Polynesia infested by *H. vitripennis* were colonized by *G. ashmeadi* within 10 months and before the deliberate introduction of the parasitoid in the islands. The parasitoid spread quickly from Tahiti to widely separated islands (up to 1400 km from Tahiti); presumably through the transportation of plant material containing parasitized *H. vitripennis* eggs, which prove the existence of failures in inter-island quarantine measures. This result demonstrated the importance of unintentional human-mediated dispersal assisting rapid and widespread distribution of an upper trophic level organism.

EFFICACY OF BIOCONTROL AGENT. (from Grandgirard *et al.* 2008a, 2008b)

In Tahiti and Moorea, the impact of *G. ashmeadi* on *H. vitripennis* populations was determined by comparing parasitism of *H. vitripennis* eggs by *G. ashmeadi* and abundance of *H. vitripennis* before and after parasitoid release at multiple paired-sites: a control (no parasitoid releases) and a release site. In addition, the abundance and distribution of *H. vitripennis* nymphs on each infested island was monitored on *Hibiscus rosa-sinensis* (Malvaceae) hedges located at different sites on the coast of each island.

Results showed that *G. ashmeadi* successfully controlled populations of *H. vitripennis* on 10 infested islands of French Polynesia across four island groups and three archipelagos (Windward and Leeward Islands [Society Islands Archipelago], Marquesas and Austral archipelagos). These islands exhibit different seasonal temperature regimens. Suppression of *H. vitripennis* populations was achieved with the release of relatively few parasitoids (~15,000), and control was widespread, rapid, and effective with > 95% reduction in pest densities on all islands colonized by *G. ashmeadi*. This level of control was achieved within 7-9 months of release of *G. ashmeadi* and is expected to be permanent based on the results of two years of survey data on the Windward Islands (Fig. 3). Possible seasonal variation in parasitism rates and natural enemy and pest densities is expected especially on

island groups (e.g., the Australs) or high elevation sites with relatively cold temperatures ($\sim 20\text{--}22^\circ\text{C}$) over the fresh and dry season (May–October).

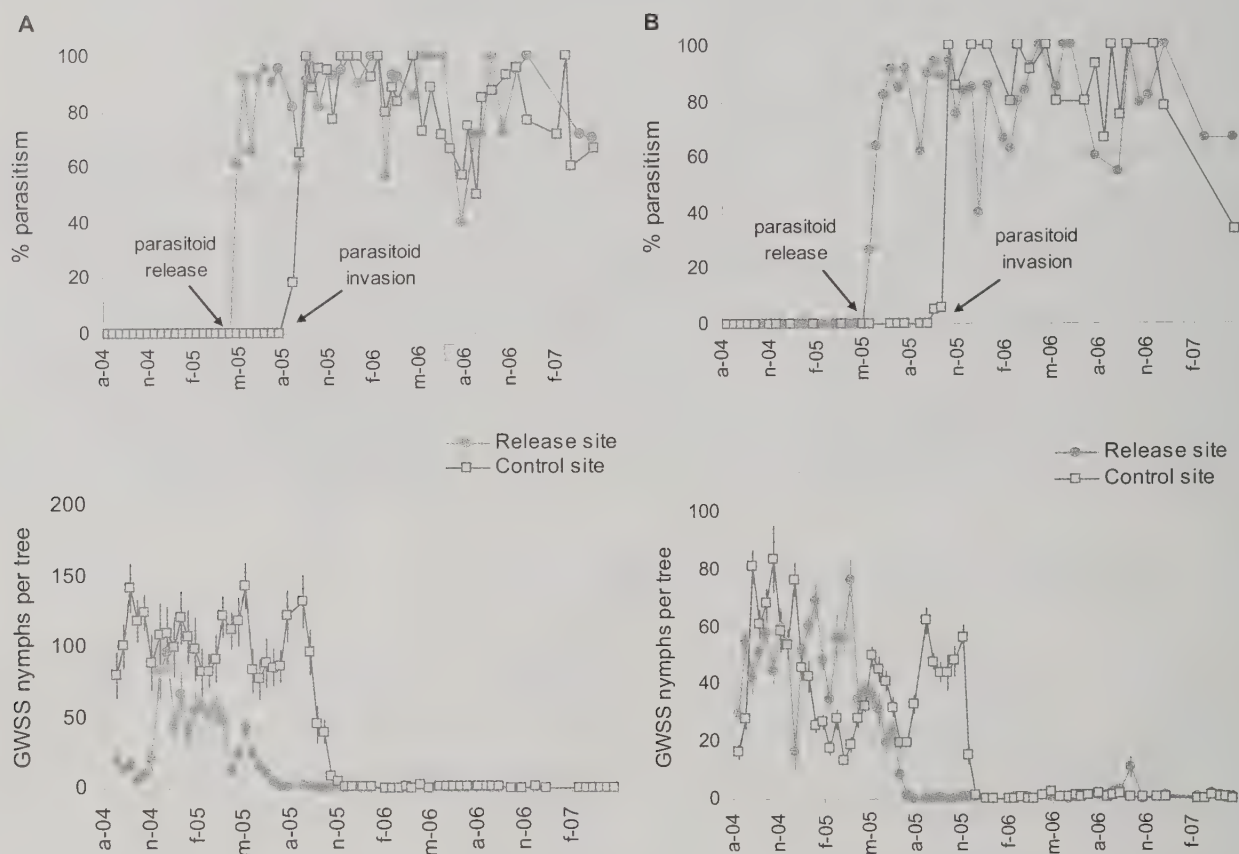


Fig. 3. Impact of *Gonatocerus ashmeadi* on *Homalodisca vitripennis* in release and control paired-sites located at sea level in Tahiti from August 2004 to April 2007. The parasitoid was released on May 2005. Upper figures: Percentage of *H. vitripennis* egg masses parasitized by *G. ashmeadi*: Lower figures: Mean number (\pm s.e.) of *H. vitripennis* nymphs found per tree during 2.5 min of visual counting: **A:** on ornamental *Scaevola* sp. in public gardens, **B:** on *Gardenia tahitiensis* in plantations. The 10 same plants were sampled each time (from Grandgirard *et al.* 2008b).

DISCUSSION.

The classical biological control program developed against the glassy-winged sharpshooter in French Polynesia demonstrated that both *H. vitripennis* and its parasitoid *G. ashmeadi* proliferate and spread extremely rapidly due to highly favourable conditions (mild climate, abundance of hosts, no specific enemies, few competitors), human-mediated transportation, in addition to high dispersal and high capacity for reproduction. French Polynesia was a paradise for *H. vitripennis* and became a paradise for its enemy *G. ashmeadi*. This program showed that classical biological control against invasive species can work and be safe. It appears as a promising method in tropical islands which have permissive environmental conditions

and can be rapidly colonized by the released biocontrol agent due to the small size of the islands.

However, it should be kept in mind that classical biological control is not a perfect solution against invasive species. In fact, there is no perfect solution against once invaders arrived; the best solution remains prevention. Hence, the cost/benefit balance of each possible control option should help deciding the best method for each invasive species. For example, the other control option against *H. vitripennis* was chemical control, however, the wide distribution of the pest (including inaccessible zones) made the chemical control option too dangerous for biodiversity and people and too expensive while classical biological control appeared feasible with low risk to the environment.

The advantages of classical biological control are its low cost, its permanency, and the absence of pollution. The drawbacks are also worth understanding:

1. The invader will never be completely eradicated, consequently, it can continue to spread and can still be a serious pest (for example if it is a disease vector and it requires a low density to infect numerous plants). In the case of *H. vitripennis*, new infested islands were discovered even after the sharpshooter was controlled efficiently by the parasitoid (inside French Polynesia, and in neighbouring countries: Easter Island, Cook Islands). The parasitoid has moved with its host in most cases and found appropriate climate to reproduce, hence *H. vitripennis* remained at low level. But it might not be the case if *H. vitripennis* begin to spread in New Zealand or Australia;

2. The introduced natural enemy may attack the native fauna and threaten the biodiversity and if this occurs, the ecosystem will be indefinitely modified. The selection of the natural enemy to be introduced is a crucial step and it is absolutely necessary to carry out broad safety studies prior to natural enemy introduction, especially in islands that have high endemism. Ideally, non-target impact studies should occur over a suitable geographic range that could climatically accommodate the natural enemy. For *G. ashmeadi*, the appropriate area of concern for potential non-target impact assessment studies could have included the central and eastern Pacific. However, this increased range would have greatly increased the cost and scope of the *H. vitripennis* biological control program to the point of infeasibility. Curtailment of the biological control program would have allowed the pest to continue its spread from highly infested areas into new areas of the South Pacific (Petit *et al.* 2008c). Hence, it is important to consider the cost/benefit balance of accumulating risk assessment data vs. continued damages and spread of the invader.

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THE RED PALM MITE, *RAOIELLA INDICA*: EFFECT OF RESIDENT AND COMMERCIALY PRODUCED PREDATORS AGAINST A RECENTLY INTRODUCED PEST IN FLORIDA, USA

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ABSTRACT.

The red palm mite, *Raoiella indica* Hirst invaded the Caribbean in 2004 and Florida, USA during the last month of 2007. Previous to the arrival of *R. indica* in Florida, surveys were undertaken to determine the current beneficial fauna inhabiting plant hosts of *R. indica*. Predators present included, *Amblyseius largoensis* (Acari: Phytoseiidae), *Bdella distincta* (Acari: Bdellidae), *Stethorus utilis* (Coleoptera: Coccinellidae) and *Chrysoperla* spp. (Neuroptera: Chrysopidae) among others. After the arrival of *R. indica*, predaceous fauna have been dominated by *A. largoensis* representing 77.2% of the total predators collected, followed by *Aleurodothrips fasciapennis* (Franklin) (Thysanoptera: Phlaeothripidae) (20%), while other predators (i.e., *B. distincta*, *S. utilis* and *Crysoperla* spp.), have been found less frequently. No significant correlations have been determined until now between the population levels of natural enemies and population levels of *R. indica* ($F_{1,3} = 2.36$; $P = 0.21$; $F_{1,2} = 1.3$; $P = 0.49$) for Broward and Palm Beach, respectively. Preliminary tests of the efficacy of the predator, *Amblyseius swirski*, show some promise, but further tests are necessary to determine its role for the management of the red palm mite.

INTRODUCTION.

The red palm mite, *Raoiella indica* Hirst. (Acari: Tenuipalpidae), also known as the coconut mite (Somchoudhury & Sarkar 1987), coconut red mite (Jalaluddin & Mohanasundaran 1990), red date palm mite (Elwan 2000), leaflet false spider mite (FAO 2005), frond crimson mite, scarlet mite (Gassouma 2005) is an important pest of coconuts, date palm, other palm species (PROSEA 2006), and bananas, beans, and durian in different parts of the world. Previous to its arrival in the New World, the mite was found in India, Philippines, Mauritius, Reunion, Malaysia, Israel and Egypt. *Raoiella indica* was found in Martinique and St. Lucia in 2004. During 2005, the mite was found in Dominica and during 2006 on the islands of Trinidad and Tobago, Guadeloupe and Saint Martin (Kane et al. 2005; Etienne & Fletchmann, 2006), and in Puerto Rico (Rodrigues et al. 2007). The red palm mite was discovered in Florida during December 2007 (Peña et al. 2008). Damages to coconut, ornamental palms and bananas are extensive. It is considered that the pest has serious consequences for the coconut, ornamental palm and banana industries of the Caribbean islands. Damage to coconuts results in a 70% yield reduction and possibly job losses leading to a major socio-economic problem for some of the islands (Mr. Philippe Agostini, President Trinidad and Tobago Coconut Growers Assoc., Pers. Comm). In Florida,

cost of regulatory actions, such as precautionary sprays before shipping *R. indica* hosts to other states, will represent an additional production cost of more than half a million dollars to palm nursery producers per year.

Raoiella indica is found on the underside of the leaves of the host plant in very large numbers. Attacked leaves display severe yellowing followed by necrosis. Heavy mite infestations result in death of young plants. Management programs for this mite in areas where it is present as well as preventative programs for areas where it has not yet arrived are urgently needed. Past chemical control tactics against *R. indica* were proven inefficacious and costly in India and Egypt, but they are considered necessary for regulatory purposes in infested palm exporting areas (Florida & Puerto Rico).

Biological control is one of the most important alternatives to conventional pesticide use in pest management. Biological control is free of many problems associated with pesticide use, such as pest resistance, environmental pollution, and worker health impacts. Classical biological control, or the introduction of natural enemies from the areas of origin for *R. indica* has been initiated under the auspices of APHIS, PPQ (Marjorie Hoy, Pers. Comm.), resulting in the quarantine screening of one classical biocontrol candidate. Prior to release of any exotic predator, it is necessary to put greater emphasis on other areas of biological control, such as natural enemy conservation and augmentation.

In India, during a survey for indigenous predators, several predators were discovered preying on *R. indica*. The phytoseiid mite, *Amblyseius channabasavanni* and a beetle, *Stethorus keralicus* Kapur (Coleoptera: Coccinellidae) were considered to be the most important predatory species (Daniel 1981). Puttaswamy & Rangaswamy (1976) cite *S. keralicus* feeding throughout the year on *R. indica* infesting coconut and areca palms in India. In the UAE, Gassouma (2005) indicates that there is natural control for the red palm mite, but the author does not report the names of the natural enemies responsible for this type of control. The biology and habits of *A. channabasavanni* were determined by Daniel (1981). He determined that *A. channabasavanni* females consumed eggs and female host mites. Alternate food sources in the field included *Tetranychus fijiensis*, eggs and crawlers of scale insects and mealybugs that infest arecanut leaves. The field population of the predator was maximal during May to June when the prey was at its peak.

Moutia (1958) observed that in Mauritius the principal predator of *R. indica* in coconut plantations was *Typhlodromus caudatus* Chant (*Amblyseius caudatus* Berlese). Gupta (2001) cites *Amblyseius longispinus* (= *Neoseiulus longispinus* Evans 1952) (Acari: Phytoseiidae) and *Stethorus parcempunctatus* and *Jauravia* sp. (Coleoptera: Coccinellidae) in the area of Karnanka while in the area of Kerala, the prevalent predators are *A. channabasavannai* and *Stethorus tetranychii*.

The objectives of this study were 1) to determine the density of predators and herbivorous micro-arthropods associated with palms and bananas in Florida before the arrival of *R. indica* and record the response of the current beneficial fauna to the invading mite and to 2) initiate preliminary tests to determine which commercially available predators will reproduce, feed and survive on a *R. indica* diet.

MATERIALS AND METHODS.

Generalist fauna prior to invasion by the red palm mite.

A survey for natural predators infesting palms and bananas was conducted in South Florida where most of the palm, *Musa*, gingers and heliconid production is concentrated. Monthly surveys were conducted in Miami-Dade and Monroe counties on coconut palms and on bananas. Twenty pinnae per frond were collected per palm species while 20 sections (ea. $\sim 60\text{ cm}^2$) were removed from a banana leaf. Samples were taken to the laboratory, where microarthropods, i.e., tetranychids, tenuipalpids, diaspidids, phytoseiids, bdellids and others were recorded, labeled and mounted and sent for identification by specialists, and densities of these spp. determined.

Naturally occurring generalist predators currently preying on *R. indica* in Florida.

Eight coconut palms were selected in two areas, West Palm Beach ($26^{\circ}42'54''\text{N } 80^{\circ}02'22''\text{W}$) and Broward ($26^{\circ}07'28''\text{N } 80^{\circ}14'58''\text{W}$). Once each month, since the time of the discovery of the *R. indica* invasion on December 2007, 1 pinna was collected from each of two fronds per palm totaling 16 samples per site. Each pinna was placed in a plastic bag, sealed and placed in a refrigerated cooler, and frozen before evaluation. Each sample was evaluated under the microscope, and number of red palm mite, tetranychids, diaspidid scales, whiteflies, mealybugs and different predaceous arthropods (Phytoseiidae, Neuroptera, Thripidae, Coccinellidae) recorded.

Potential efficacy of commercial predators for control of *R. indica*.

Several commercially produced predators will be tested for efficacy to feed, survive and reproduce on *R. indica* (i.e., *Amblyseius swirski*, *Phytoseiulus persimilis*, *Neoseiulus longipes*, *N. californicus*, *Galendromus occidentalis*). The predator, *Amblyseius swirski* was selected for a preliminary trial under laboratory conditions. The arena consisted of a 5 cm petri dish placed inside of a 12 cm Petri dish. Water was added to the larger Petri dish to prevent predator or prey escape from the arena. A 4 cm^2 section of a coconut frond that held a known number of different stages of *R. indica* was added. Then, a presumably-mated 1 to 2 day old single female was individually placed inside of each arena. The numbers of eggs, nymphs and adults consumed daily were calculated and new food sources added daily.

Preliminary field trials with a commercial predator.

Five coconut palms infested with *R. indica* were selected. Ten pinnae from different fronds were collected per palm and the number of *R. indica* and predatory phytoseiids recorded from a 100 cm^2 section of a each pinna. Then, a Swirski-mite plus® sachet containing approximately 250 predators was hung from the middle canopy of each palm. *R. indica* and phytoseiid densities were assessed 1 month after.

RESULTS AND DISCUSSION

Generalist fauna prior to invasion by the red palm mite.

The scale, *Aonidiella orientalis* (Newstead) (Heteroptera: Diaspididae), the whitefly, *Aleurocanthus woglumi* Ashby (Heteroptera: Aleyrodidae), and the spider mites, *Tetranychus* spp., and *Tetranychus gloveri* Banks (Acari: Tetranychidae), were the most common microarthropods inhabiting coconuts, while *Tetranychus* sp., and *Brevipalpus* spp., were the most common inhabiting bananas from 2006 to 2008 in areas before the arrival of *R. indica* (Figs 1 and 2). On coconuts, *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), *Stethorus utilis* (Horn) and *Chrysoperla* spp., (Neuroptera: Chrysopidae) were the most common predators followed by *Bdella distincta* (Barker and Bullock) (Acari: Bdellidae) and *Aleurodothrips fasciapennis* (Franklin) (Thysanoptera: Phlaeothripidae). On bananas, the predators *Amblyseius* spp., and *Stethorus utilis* were recorded (Figs 3 and 4). *Amblyseius largoensis* was the only phytoseiid on coconuts, while the species identification of the *Amblyseius* found in bananas is pending. *Amblyseius largoensis* is a generalist feeding not only on mites, but also on pollen (Yue & Tsai 1996). A study conducted in Brazil to determine survival of *A. largoensis* on *Aceria guerreronis* (Acari: Eriophidae), a pest of coconuts, determined that a mix diet of *A. guerreronis*, or *T. urticae* + pollen and + honey increased its fertility parameters (Galvao et al., 2008). *Amblyseius largoensis* was one of two predators found in Philippines in association with *R. indica* (Gallego et al., 2002), but its efficacy has not been determined. *Amblyseius largoensis* has been also found in association with *A. guerreronis* on coconuts fruits affected with the eriophyid, but no clear prey preference has been determined for this predator on this plant (Reis et al. 2008). The predaceous thrips, *A. fasciapennis* has been previously reported feeding on eggs of diaspidid scales such as *Chrysomphalum aonidum* and *Aspidiotus nerii* and on eggs of the pyralid moth, *Corcyra cephalonica* (Watson et al., 2004; Beshear & Nakahara 1975). *Stethorus utilis* is cited as a common predator of tetranychid mites (Chazeau 1985). In general, because of the high densities of both diaspidids and aleroydids present on coconuts, the prey preference for the native predators needs further study under Florida conditions.

Naturally-occurring generalist predators currently preying on *R. indica* in Florida.

In the area of Palm Beach, where early colonization of *R. indica* is suspected, a total of 217,622 mites were collected per pinna between January and June, 2008; average *R. indica* densities fluctuated between 1000 to 1200 per pinnae during the winter months (January –February) and peaked during March (approx. 4,000), with a slight reduction during the following months (April to June) (Fig 5). A total of 166 *A. largoensis* were collected between January-June 2008. *A. largoensis* represented 77.2% of the total predators collected, followed by *A. fasciapennis* (20%), while other predators (i.e., *B. distincta*, *S. utilis* and *Crysoperla* spp.), were only a small proportion of the total (2.8%).

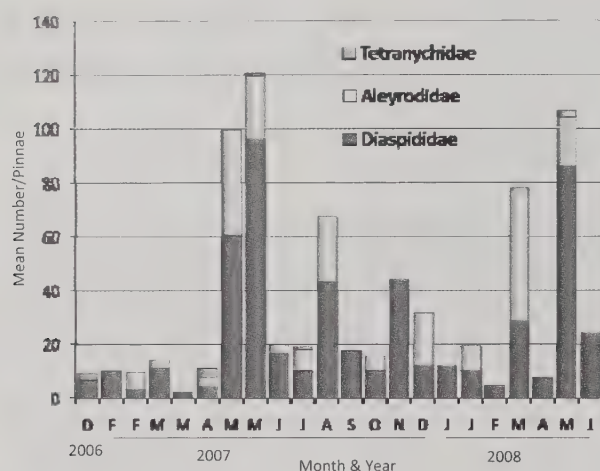


Fig. 1. Dynamics of herbivorous micro-arthropods inhabiting coconuts prior to establishment of *R. indica* in south Florida (Miami Dade).

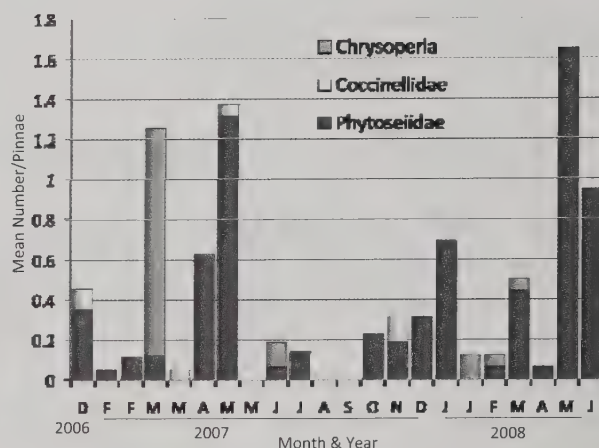


Fig. 2. Dynamics of natural enemies inhabiting coconuts prior to establishment of *R. indica* in south Florida (Miami Dade).

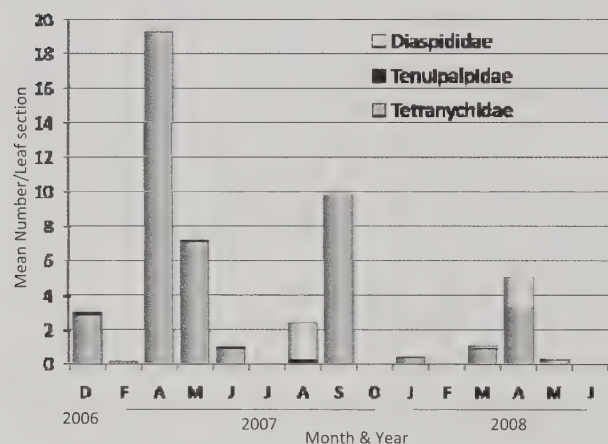


Fig 3. Dynamics of herbivorous microarthropods inhabiting bananas prior to establishment of *R. indica* in South Florida (Miami-Dade)

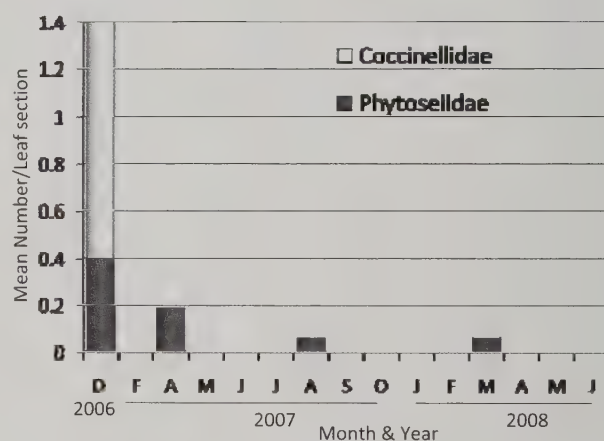


Fig. 4. Dynamics of natural enemies inhabiting bananas prior establishment of *R. indica* in South Florida (Miami-Dade)

The relation between phytoseiid predators, i.e., *A. largoensis* and the potential new prey was erratic. For instance, while an increase on predator density was observed following an increase on the prey during January-February, and May -June, 2008, numbers of predators were still consistently low, compared to the number of prey present. In the area of Broward, a total of 45,280 red palm mites were collected per pinna between January and June, 2008; This is a much lower density than that of Palm Beach. Average *R. indica* densities fluctuated ca. 100 to 1,600 mites per pinnae (Fig 6). A total of 22 *A. largoensis* was collected between January to June, 2008 in that area. An increase of predator density was not observed until the month of June, approx. 6 months after the detection of the pest.

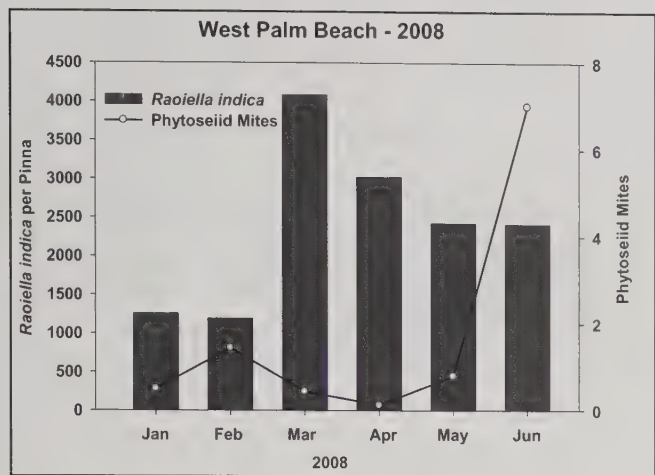


Fig 5. *Raoiella indica* population and phytoseiid densities after the invasion of the red palm mite In West Palm Beach, FL, USA

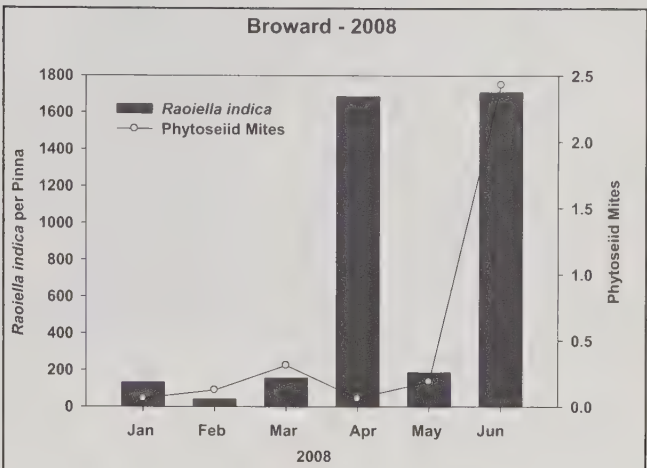


Fig 6. *Raoiella indica* population and Phytoseiid densities after the invasion of the red palm mite in Broward, FL, USA

No significant correlations were found between the population levels of natural enemies and population levels of *R. indica* ($F_{1,3} = 2.36$; $P = 0.21$; $F_{1,2} = 1.3$; $P = 0.49$) for Broward and Palm Beach, respectively. While we have not reached one year from the discovery of the infestation of *R. indica* in Florida, at this point, it is doubtful that the predator, *A. largoensis* will be capable of maintaining the prey under lower densities than those observed until now (Figs 5 and 6). No significant correlations between abiotic factors (temperature, precipitation) and the population levels of *R. indica* were observed during these months at either site (Temperature: $F_{1,3} = 5.83$; $P = 0.09$ for Broward and $F_{1,3} = 1.70$; $P = 0.28$ for Palm Beach. Precipitation: $F_{1,3} = 0.08$; $P = 0.79$ for Broward and $F_{1,3} = 1.75$; $P = 0.27$ for Palm Beach).

Potential efficacy of commercial predators for control of *R. indica*.

A female of *A. swirski* consumed a daily average of 7.06 ± 0.61 eggs, 2.49 ± 0.21 larvae, 3.25 ± 0.29 nymphs and 8.90 ± 0.55 adults of *R. indica*. Female longevity was 11.54 ± 0.72 days with an average daily oviposition of 1.1 ± 0.10 eggs per day. It is not known what would be the predation potential of immatures of this species on *R. indica*.

Preliminary field trials with a commercial predator.

A significant reduction on *R. indica* was observed 1 month after release of 250 *A. swirski* per palm in the area of Manalapan ($F_{1,18} = 13.66$, $P = 0.001$). However, no significant increases on phytoseiid densities were recorded (Fig 7). Further studies are needed to determine the effectiveness under field conditions of *A. swirski* as a predator of *R. indica*.

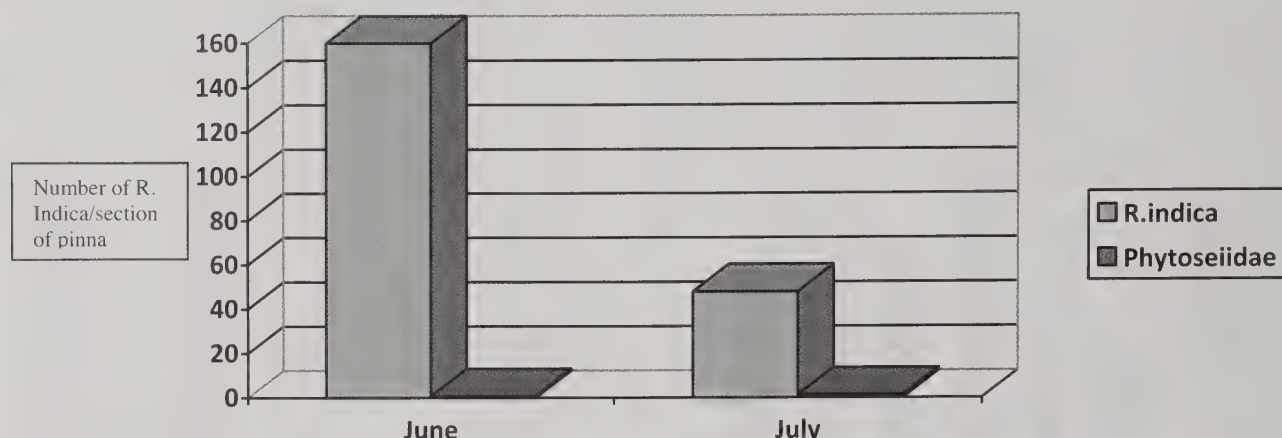


Fig. 7. Monthly average of *R. indica* densities before and after release of *A. swirski*, Palm Beach, FL, 2008.

The beneficial fauna inhabiting coconut palms in Florida is diverse and its numbers have increased after the arrival of the red palm mite, *Raoiella indica*. This increase, although significant, has not substantially reduced the density levels of the red palm mite. Other tactics, either focusing on augmentation or on introduction of other predators should be the next step to promote effective biological control of the mite.

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THE USE OF CLASSICAL AND AUGMENTATION BIOLOGICAL CONTROL FOR THE SOUTH-EAST ASIAN BORER *CHILO SACCARIPHAGUS* BOJER (LEPIDOPTERA: CRAMBIDAE) IN MOZAMBIKAN SUGARCANE

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ABSTRACT.

In 1999, *Chilo sacchariphagus* (Lepidoptera: Crambidae) was recorded from sugarcane west of Beira, Mozambique. The following year it was recorded north of Beira, from an estate on the Zambezi River. Using mitochondrial DNA analyses, the infestation was identified as originating from Mauritius, where sugarcane is also affected by this borer. Initial surveys revealed the presence of an effective indigenous trichogrammatid egg parasitoid (*Trichogramma bournieri*). In contrast, larval and pupal parasitism was extremely low. A classical biocontrol programme using the Ichneumonid *Xanthopimpla stemmator* was initiated, and a population established on pupae of the host at both sugar estates. This left a niche open for the classical introduction of a larval parasitoid. The braconid *Cotesia flavipes* has been used successfully in many parts of the world to control species belonging to the genus *Chilo*. In south and eastern Africa, a very successful classical biocontrol programme using this parasitoid against the maize stalk borer *Chilo partellus* has been implemented. In 2004, this parasitoid was recorded from *C. sacchariphagus* at the northern-most sugar estate. Mitochondrial DNA analyses showed specimens recovered were the population released against *C. partellus* in maize in Africa. The question was thus asked, could this parasitoid population be augmented following its host plant and host insect shifts? This paper discusses the results of these studies in the light of reducing infestation on the affected estates, and preventing its spread into subsistence fields and new estates being established for biomass production in the area, as well as sugarcane estates in surrounding countries.

INTRODUCTION.

Chilo sacchariphagus Bojer (Lepidoptera: Crambidae) is indigenous to south-east Asia, where it is part of a complex of stalk borers attacking sugarcane in particular (Cheng, 1994; Kuniata, 1994). It has been introduced into the Mascarene Islands and Madagascar, the sugar industries of which have implemented classical biocontrol to restrict its impact on their industries (Williams, 1983; Goebel, 1999). Although its presence on mainland Africa was reported from Mozambican sugarcane in 1989 (van Rensburg *et al.*, 1989), its identity was only confirmed in 1999 (Way &

Turner, 1999), from Acucareira de Moçambique (A de M) Sugar Estate, Mafambisse (34° 10'E; 19° 20'S) just inland from Beira. In 2001 it was found at Companhia de Sena (Sena), Marromeu (18°17'S; 35°57'E; 6-11 amsl) a sugarcane estate further north, on the Zambezi River (Conlong & Goebel, 2002). Thus far it has not been found in any of the other sugar estates in Mozambique nor in African countries bordering Mozambique. This may not be the case in the future though, as sugarcane expansion, for sugar and biomass production, in the area from which *C. sacchariphagus* presence has been confirmed has expanded considerably, particularly in the directions of Zimbabwe, Malawi and Tanzania.

The South African Sugarcane Research Institute (SASRI) became involved in biological control research against *C. sacchariphagus* in 2000, when the management of A de M requested that a biological control programme be implemented against *C. sacchariphagus* on their estate, in 2001, the management of Sena requested the same to be completed on their estate. At the same time, a classical biocontrol programme against another graminaceous borer of south-east Asian origin, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), spearheaded by the International Centre for Insect Physiology and Ecology (ICIPE), Kenya was underway (Overholt *et al.*, 1994), with activities spreading to the maize production areas of Mozambique (Cugala & Omwega, 2001; Cugala *et al.*, 2001), some of which overlapped with the sugar producing areas.

This paper reviews the progress of the programme against *C. sacchariphagus* in Mozambican sugarcane, and how in the more recent years it has become more integrally involved with the ICIPE programme against *C. partellus*. These results are then discussed in the context of host plant, and host insect shifts, and their implications for successful biological control of *C. sacchariphagus* in Mozambican sugarcane.

LOCAL INDIGENOUS PARASITOIDS.

Conlong & Goebel (2002) report on the first field surveys completed at A de M, the impact of *C. sacchariphagus* on their cane, and the impact of indigenous parasitoids found at that time on the *C. sacchariphagus* population. At that time, minimal indigenous parasitism of larval and pupal life stages of *C. sacchariphagus* was found. However, egg parasitism was abundant.

Parasitoids emerging from parasitized egg batches were sent to INRA, Antibes, France (Entomology and Biological Control Unit), who identified them using morphological characteristics as *Trichogramma bournieri* Pintureau and Babault (Hymenoptera: Trichogrammatidae). Voucher specimens are housed at the Natural History Museum in Paris, France (Conlong & Goebel, 2006). More intensive surveys at both estates followed, which showed that the eggs of *C. sacchariphagus* were heavily parasitized on the abaxial and adaxial surfaces of the green sugarcane leaf blades where they were oviposited in batches. In addition, complete parasitism of whole batches was very evident. It thus appears that *T. bournieri* attacking *C. sacchariphagus* in sugarcane in this new association is a good strain, with high parasitism levels recorded, and thus is probably well suited to its host- a desirable character for a species being considered for augmentation (Smith, 1996, Haile *et al.*

2002). There is a period at both estates when natural populations of *T. bournieri* are low (October and November), even though *C. sacchariphagus* oviposition was not (Conlong & Goebel, 2006). This points to a period when populations of *T. bournieri* could be augmented with laboratory-reared individuals. They accept eggs of the factitious host *Galleria mellonella* L. (Lepidoptera: Pyralidae) that is now widely mass reared (Conlong & Goebel, 2006).

The few larval parasitoids found were restricted to *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae), and the solitary braconid *Stenobracon* sp., and no pupal parasitoids were evident (Conlong & Goebel, 2002) in these surveys and in subsequent surveys at both estates.

CLASSICAL BIOLOGICAL CONTROL.

Pupal parasitoids.

Because SASRI had imported and was mass rearing *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) as a possible new association pupal parasitoid for the control of *Eldana saccharina* Walker (Lepidoptera: Pyralidae), this parasitoid was used first in the classical biocontrol programme against *C. sacchariphagus* in Mozambique. Conlong & Goebel (2002) describe the releases and results of this parasitoid at both A de M and Sena. Establishment of the parasitoid on field collected pupae of *C. sacchariphagus* was confirmed by having adult parasitoids emerging from parasitized pupae, and adult parasitoids collected in malaise traps set up in release fields a year after releases were terminated (Conlong & Goebel, 2002).

At the same time, Cugala was releasing *X. stemmator* obtained from laboratories colonies established at ICIPE from material supplied by SASRI and India, at various localities throughout Mozambique (but not at Tica, as the release site was taken as A de M, where the releases were done by SASRI). Table 1 lists these localities and the results of releases.

The significant high level of parasitism recorded in 2004 on *C. partellus* at Tica, where no releases of *X. stemmator* had been made in maize, might be due to the parasitoid's spread from the releases sites on sugarcane at A de M (About 25km from Tica) made in 2001 against *C. sacchariphagus* (Conlong & Goebel, 2002). *Xanthopimpla stemmator* emerged from *C. partellus* pupae collected on maize surrounding sugarcane fields at A de M in 2001 and 2002.

This apparent host plant shift in the A de M/Tica region from sugarcane to maize, and host insect shift from *C. sacchariphagus* to *C. partellus* shows that this parasitoid is not specific to either host plant or host insect. This bodes well for establishing reservoirs of it when either of the graminaceous crop host is not available for any reason. Cugala (unpublished results) has recently collected this parasitoid from indigenous grasses in the vicinity of maize fields in recent biodiversity surveys.

Table 1. Number of *C. partellus* and *Busseola fusca* Fuller (Lepidoptera: Noctuidae) pupae collected and level of parasitism due to *X. stemmator* on *C. partellus* pupae collected from maize in Mozambique

Locality	Mean number <i>C. partellus</i> Pupae	Mean number <i>B. fusca</i> pupae	Mean pupae per plant	Mean number of <i>X. stemmator</i> recovered	% parasitism on <i>C. partellus</i>
Guija	82±0.96a	0	0.21±0.19ab	9±0.51abc	11.0±3.51a
Lichinga	4±0.37b	15±0.65a	0.05±0.12c	0.0±0.0c	0.0±0.0c
Magude	113±0.86a	0	0.28±0.18a	12±0.54ab	10.6±3.28a
Manica	45±0.84a	18±0.69a	0.16±0.17b	2±0.27bc	4.4±1.79b
Nhamatanda	96±0.55a	0	0.24±0.12ab	5±0.40abc	5.2±2.78b
Tica	122±0.60a	0	0.31±0.13a	15±0.55a	12.3±2.95a
Xai Xai	118±0.54a	0	0.30±0.12a	10±0.49ab	8.5±2.36ab
<i>Df</i>	6, 399	6, 39	6, 399	6, 399	6, 399
<i>F</i>	23.8	2.4	12.1	4.6	6.3
<i>P-values</i>	<0.0001	0.1048	<0.0001	<0.0001	<0.0001

Numbers followed by the same letter in the same column are not statistically different (SNK, P<0.05)

Larval parasitoids.

In all surveys in sugarcane at A de M, from Conlong & Goebel (2002) until 2005, very little larval parasitism was recorded. This revealed a niche that could be exploited by a known classical biocontrol agent of *Chilo* spp., such as *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), should it be able to adapt to the conditions at A de M and Sena. This was demonstrated with the very successful classical biocontrol programme using a strain of *C. flavipes* imported from Pakistan against *C. partellus* initiated by ICIPE (Overholt et al. 1994), allowing its expansion into Mozambique (Cugala & Omwega, 2001; Cugala et al. 2001).

In 2004, in surveys conducted by the Mauritian Sugarcane Research Institute (MSIRI) at Sena, it was reported that parasitism of *C. sacchariphagus* larvae by *C. flavipes* had been recorded (S. Ganeshan, pers. comm.). Dr G. Prinsloo of the South African National Collection of Insects, Pretoria, confirmed the species identification. Mitochondrial DNA (mtDNA) analyses provided additional evidence that the adult parasitoids that emerged were *C. flavipes*, and not the indigenous *C. sesamiae* (K. Muirhead, pers. comm.). Further, in a study to determine the origin of a population of *C. flavipes* collected from *C. partellus* attacking Ethiopian sugarcane, Assefa et al. (2008) showed that African populations of *C. flavipes* sampled from six countries

(Including Mozambique) had matching mtDNA fragments. This study further revealed that the genetic differentiation within the species as a whole varied greatly. This was in contrast to the lack of genetic differentiation among African populations of *C. flavipes*, which suggested that they were all derived from the same founding population, i.e. the maize/sorghum strain of *C. flavipes* reared by ICIPE and released in different African countries against *C. partellus* in the 1990s. Furthermore, the Mozambican (and other African) specimens of *C. flavipes* were closely related to the Indian and Pakistan populations of the parasitoid and significantly different from the populations in the neighbouring Indian Ocean islands (Assefa et al. 2008), which means that the population found at Sena is not from these islands. Assefa et al. (2008) showed that populations from this Indian Ocean region are closely related to populations from Indonesia and Papua New Guinea, and different from populations in Africa. The study thus suggests that strains of *C. flavipes* are less host-specific and habitat-specific than previously thought (eg Mohyuddin, 1971; Mohyuddin et al, 1981; Inayatullah, 1983; Mohyuddin, 1991).

In the classical biocontrol programme against *C. partellus* in maize in Mozambique, *C. flavipes* was not released close to the Sena sugarcane recovery site (Cugala & Omwega, 2001; Cugala et al. 2001). The closest release site was in fact in neighbouring Malawi, on the Shire River (Schulthess pers. comm.) that flows into the Zambezi River on which Sena is situated. However, distance from release sites is not a major criterion, as Assefa et al. (2008) have shown in Ethiopia (where no releases of this parasitoid had taken place), in surveys in 2003 and 2004, that this maize/sorghum strain of *C. flavipes* was found in sugarcane fields more than 2500 km away from the 1993 release site in southern Kenya, and more than 2000 km from the 1997 release site in Somalia.

CONCLUSIONS.

It is clear that biological control of the exotic *C. sacchariphagus* at affected sugarcane estates in Mozambique is very possible and will result in reduced populations of the borer in infected sugarcane stalks should biocontrol be implemented. Avenues available are either through augmentation of the indigenous, new association egg parasitoids; classical biocontrol using imported larval and pupal parasitoids of *C. sacchariphagus*; or a combination of both.

Trichogramma bournieri has all the attributes of a very effective augmentation candidate. It is already very effective at most times of the year and populations can be augmented from laboratory colonies reared on factitious hosts such as *G. mellonella*, in the spring period of October/November when numbers of *T. bournieri* are low on the many *C. sacchariphagus* egg clumps available. Under no circumstances should the introduction of other species of egg parasitoid be considered, as they may reduce the effectiveness of this indigenous species.

Expensive explorations in the area of origin of *C. sacchariphagus* for natural enemies, especially those of its larvae may not be necessary now that *C. flavipes* has been found at Sena estate. What is needed now is regular monitoring of the estate to assess the extent of colonisation of *C. sacchariphagus* by *C. flavipes*. The colonisation could be increased by augmenting the newly establishing parasitoid population with laboratory reared individuals from reliable colonies of *C.*

sacchariphagus, or if this is not possible, *C. partellus*. The existing populations of the pupal parasitoid, *X. stemmator*, now established at the Mozambican sugar estates, could similarly be augmented with individuals reared on the latter two hosts, should reliable colonies of these be established.

By using the above approaches, combined with the use of varieties of sugarcane resistant to *C. sacchariphagus*, an effective integrated pest management (IPM) plan can be developed for all sugarcane estates in Mozambique. This can be further enhanced with habitat management to conserve the biological control agents now present on the estates, and will certainly complement sterile insect technology aimed to produce F1 sterility in the wild population.

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SESSION 4

MOLECULAR TOOLS IN BIOLOGICAL CONTROL

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Molecular diagnostic techniques have become of increasing interest in arthropod biological control programmes, and as such, this session was proposed to draw attention to the utility of such tools in a broad range of studies on parasitoid and predator ecology. Here, we bring to light some of the recent developments and applications of molecular techniques in arthropod biological control and highlight the important contributions of PCR-based tools in current and future research on natural enemies. In terms of agent identification, molecular tools have been extremely useful in separating sibling species, cryptic species, and even geographic strains of the same species – all of which are crucial to ensure the quality of mass-rearing and release efforts. Molecular tools are invaluable in the construction of parasitoid and predator food-webs, as well as in the analysis of complex trophic relationships involving natural enemies, target pest insects and the host plant(s) they feed on. Similarly, molecular tools have allowed a better understanding of the relationship between parasitoids and their endosymbionts, and how this can impact the outcome of successful biocontrol. Molecular techniques have been useful in pre-release risk assessment studies on candidate biological control agents to evaluate agent efficacy, specificity, and potential non-target effects. Similarly, the use of PCR-based techniques in post-release studies and/ or retrospective case studies has allowed confirmation of agent establishment, evaluation of agent efficacy, and analysis of the success or failure of previous biocontrol efforts. Population genetic studies documenting founder effects and genetic bottlenecks that occur following natural enemy introduction also rely on the use of molecular tools, and can provide insight into the ecological and evolutionary processes affecting classical and augmentative biocontrol. By covering a broad range of topics in this symposium, we hope to inform biocontrol practitioners of the potential applications of molecular tools in their own field, and encourage their incorporation in biological control studies to improve our understanding of and success in the biocontrol arena.

USING MOLECULAR TOOLS TO IDENTIFY AND DESCRIBE ECOLOGICAL AND EVOLUTIONARY PROCESSES AFFECTING AUGMENTATIVE BIOLOGICAL CONTROL

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ABSTRACT.

Evolutionary theory and developments in molecular biology tools have only recently begun to be applied to investigate how Classical Biological Control agents interact in their new environment. The application of molecular tools has the potential to improve biological control agent introduction, rearing, and risk assessment methodology. To our knowledge these tools have not previously been used to understand how augmentative releases of biological control agents interact with established wild populations, yet doing so would allow researchers to model gene flow and population expansion to better understand augmentative control efficacy and post-release, wild type recruitment. This study assessed the genetic structure of populations of *Diachasimorpha tryoni* released augmentatively on the island of Maui in 1988. A microsatellite library was developed from contemporary populations to elucidate inter- and intra-population structure. Due to DNA degradation only eight microsatellite loci amplified consistently in insects sampled from museum collections. Analysis of museum collections suggested that population structural changes occurred due to augmentative control practices prescribed on the island of Maui ~20 years ago. This preliminary data also implies local population recruitment and population genetic structural dilution, suggestive of a *Reverse Bottleneck* following those augmentative practices.

INTRODUCTION.

The application of augmentative Biological Control (BC) has proven an invaluable tool in the field of agricultural pest management (van Lenteren & Bueno 2003; van Lenteren 2003, 2006; Wong *et al.* 1991). Theoretically, it offers pest reduction and suppression comparable to insecticides, without the fear of excessive non-target interactions (van Lenteren 2003). Yet, by definition, augmentative release approaches, when used to augment established populations, may modify the environment by interbreeding with established populations, thereby modifying the genetic structure of wild populations within the natural environment. These modifications would theoretically be a genetic shift from locally adapted populations to populations characterized by the highly inbred and genetically depauperate insectary reared (IR) agent of choice (van Lenteren 2003). This phenomenon is indicative of genetic structural dilution of the initial (wild) population with the clonal IR population, what we coin here as a *Reverse Bottleneck*. Given that there is typically a positive correlation between population fitness and heterozygosity of loci within that

population (although there are increasing examples of clonally adapted highly invasive populations, see Le Roux *et al.* (2007)) it is possible that the effectiveness of the BC agent would be reduced by the mixing of wild type with IR populations of the agent. While forecasting the impacts of augmentatively released populations is imprecise, modeling genetic association (dispersal, gene flow, relatedness) of these populations can lead to general theories applied to agent choice and rearing methodologies (Hufbauer *et al.* 2004; Avise 2004; Roderick & Navajas 2003).

To assess what role micro-evolutionary processes play on an IR, inundatively released BC agent and its wild counterpart we chose as our model organism the arrhenotokous Opiine *Diachasimorpha tryoni*, Cameron (Hymenoptera; Braconidae). This tephritid parasitoid was successfully used in an oft-cited augmentative release program in 1988 to suppress populations of *Ceratitis capitata*, Wiedemann (Diptera; Tephritidae), in Hawaii (Wong *et al.* 1991). To evaluate efficacy, these authors sampled from two locations in 1989, a year after release - Kula, Maui where they released ~272,000 *D. tryoni* weekly for approximately 8 weeks, and Keokea, Maui, ~2 kilometers away, where no releases were made. Population fluctuations of *C. capitata* and *D. tryoni* were quantified and statistically compared between the two locations (Wong *et al.* 1991). Dried specimens from this study were stored in the Hawaii Department of Agriculture (HDOA) collections of Mohsen Ramadan. Also, pre-release specimens were obtained from the HDOA collections and the Australian National Insect Collection; these are respectively the 1986 IR population reared on Oahu and later used for the releases, and a 1959 collection of wild *D. tryoni* from Kula, Maui. Microsatellites, highly variable neutral genetic markers, were used to understand this system in relation to its geographic structure.

Given the extent and scale of these releases, (~4.1 million IR *D. tryoni* were released) we hypothesized that at the release foci, the wild population structure in Kula, Maui would be highly similar to the initial IR population even though all collections analyzed from these trials were taken one year post release (Wong *et al.* 1991). We propose that genetic displacement of wild populations would be less likely as one moves away from the foci through space and time, and that at the point of release the absolute dilution of the initial wild population would possibly lead to a *Reverse Bottleneck*, whereby the genetic structure of the wild population is highly diluted by the introduced individuals.

METHODS.

Population Sampling and DNA Extraction.

Seven putative populations were sampled giving a total of 111 individuals. All individuals used in this analysis were either dried or alcohol preserved. All but one population were from the collections of M. Ramadan at HDOA, R. Lukins collected the other population in 1959. Collection locations and other population information are given in Table 1 and Fig. 1.

A single hind leg was removed from each museum specimen. Due to the haplo-diploid nature of *D. tryoni* only females were used in this analysis. DNA was isolated and purified from each leg sample following the DNeasy extraction kit protocol (Qiagen Inc, 2006).

Table 1. Population information concerning the origin and preservation of all museum *D. tryoni* specimens used in this study. The collection from the Australian National Insect Collection are indicated by ANIC while those from the Hawaii Department of Agriculture are indicated by HDOA.

Pop	Location Coll.	Date Coll.	Collector	N	Preservation	Collection
1	Kula, Maui	8/17/1959	R. Lukins	6	Dried	ANIC
2	(IR) ; Manoa, Oahu	6/30/1986	M. Ramadan	15	EtOH	HDOA
3	Kula, Maui	1/9/1989	M. Ramadan	4	Dried	HDOA
4	Keokea, Maui	3/28/1989	M. Ramadan	4	Dried	HDOA
5	Keokea, Maui	4/12/1989	M. Ramadan	26	Dried	HDOA
6	Keokea, Maui	4/19/1989	M. Ramadan	30	Dried	HDOA
7	Kula, Maui	5/27/1989	M. Ramadan	26	Dried	HDOA



Fig. 1. Map showing the locations of interest on the islands of Maui (Keokea/Kula) and Oahu.

Microsatellite Analysis.

Eight microsatellite loci (Vorsino *et al.* unpub. data) were separated into two multiplex sets and amplified in 10 uL reaction volumes containing 4.8 µL Qiagen multiplex mastermix (HotStarTaq DNA polymerase, Multiplex PCR buffer and

dNTP's), 0.2 μ M of each primer, 0.4 μ L of ddH₂O and 2.4 μ L of template. An initial denaturation and HotStarTaq activation step of 95°C for 15 min. was followed by 40 cycles of 94°C for 30 sec., 58°C for 90 sec. and 72°C for 60 sec. the final extension was conducted at 60°C for 30 min. per the manufacturers recommendation (Qiagen Inc. 2008). All samples were genotyped on an ABI 3730XL sequencer.

Statistical Analysis.

The microsatellite loci amplified from DNA extracted from these museum specimens were genotyped, scored, and assessed for neutrality (Vorsino *et al.* unpublished data). Loci scores were then exported into Excel (Microsoft Excel 2007) for data management and imported into the freeware Convert (Ver. 1.2) for data conversion (Glaubitz 2004).

The programs STRUCTURE (ver. 2.2) (Falush *et al.* 2003) and Geneland (ver. 1.1.4) (Guillot *et al.* 2005) were used to describe population structure and gene flow of these unlinked loci in a spatial and non-spatial context (Pritchard *et al.* 2002). STRUCTURE uses a model-based Bayesian clustering approach to probabilistically assign individuals to a population (or if admixed, >1 populations) depending on their genotypic makeup (Pritchard *et al.* 2002). Geneland is an R based landscape genetics application that uses Bayesian based assignment tests to identify spatially explicit genetic discontinuities by means of Voroni tessellations (Guillot *et al.* 2005; Chen *et al.* 2007).

In STRUCTURE, parameter sets were defined that best describe the data and allow α (Dirichlet Parameter for Degree of Admixture) convergence. A burn-in period (for reliability) of 100,000, in combination with a run length of 100,000 Markov Chain Monte Carlo (MCMC) was performed. We implemented the admixture model to define population structure since individuals were assumed to have mixed ancestry due to recent immigration (Falush *et al.* 2003). We chose to run the analysis in the dependent structure analysis mode because it assumes that allele frequencies are likely to be similar due to recent divergence and would thus fit our data appropriately. In the dependent population structure analysis seven populations were defined through prior probability estimates for defining genetic population structure (K) independent of assumed estimates of K (Pritchard *et al.* 2002; Falush *et al.* 2003).

Geneland was used to elucidate the population membership of each individual in a spatial context (Guillot *et al.* 2005, 2008). In order to incorporate this analysis we first needed to define the geographic coordinates for each individual within their defined collection zones (i.e. Kula and Keokea). Coordinates were randomly selected in those spatial zones to identify individuals and genotype membership from those zones. This was necessary as the exact geographic positions of the collections were not given and separation of individuals to define and understand population membership was essential. Geneland, parameters were initially set to identify K using posterior probability of membership with 100,000 iterations, combined with 100 independent runs, a bootstrap of 499 (the maximum possible) was used on each independent run (Guillot *et al.* 2008). The value of K was identified as the highest posterior probability given out of all the independent runs as recommended by Guillot *et al.* (2008). The allele frequency model was set to "correlated" (much like admixture), with a spatially false organization (Chen *et al.* 2007). Because only the zonal locale of the individuals was known, not the exact coordinate of collection, the

uncertainty of each coordinate was set to 40% to better visualize possible overlapping samples.

RESULTS.

Bayesian Analysis.

The STRUCTURE output allowed us to assess population membership and gene flow in *D. tryoni* (Fig. 2). We re-visualized the output to reveal genetic structure of populations in relation to individual population membership, thereby removing any assumptions of geographic origin through the estimated membership coefficient (Q). This is similar to the methods of Beaumont *et al.* (2001) in their approach to defining population structure and membership, dependent on assumptions other than geographic origin, and in the use of a wildtype genetic structure (1959 Kula) to better understand population membership. Population membership was based on ~40% cluster membership for each individual given the high probability of wild X laboratory reared crosses.

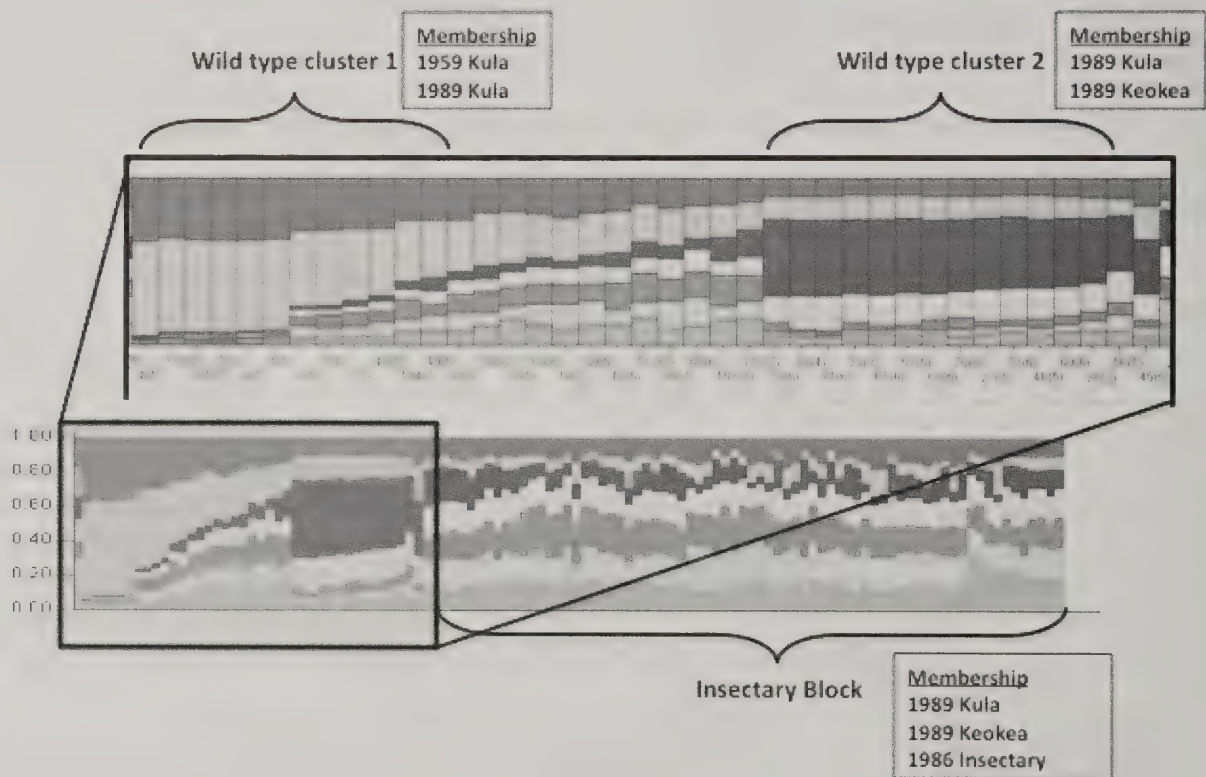


Fig. 2. STRUCTURE output indicating wild type and IR clusters with membership based on Q. This figure indicates admixed wildtype clusters from both Kula and Keokea with 1959 population clusters of Kula being the absolute wild-type outgroup (in this type of analysis it is referred to as the learning sample) (Beaumont *et al.* 2001). The IR block populations are comprised of most of the IR individuals measured, as well as Kula and Keokea individuals from 1989. This block showed equal membership of each individual into all the clusters and is an indication of admixture (Mullen & Hoekstra 2008).

Initial analysis of all the populations in Geneland (1956 – 1989) indicated a K value ~ 8 (Fig. 3A), suggesting eight clusters. The population membership map (Fig. 3B) is representative of the consolidated Voronoi tessellations (divisions of a specified area into polygonally defined regions based on relatedness) shown below the map (Guillot *et al.* 2005). The emphasized area is of the Kula and Keokea regions and is indicative of both IR based and unique clusters. The 1959 Kula data is emphasized as one of those unique clusters. The tessellations also cluster relationships, whereby a color gradient defines degree of association. The gradient is from white, which represents individuals that fall directly within that cluster, to red, representative of no association (Guillot *et al.* 2005).

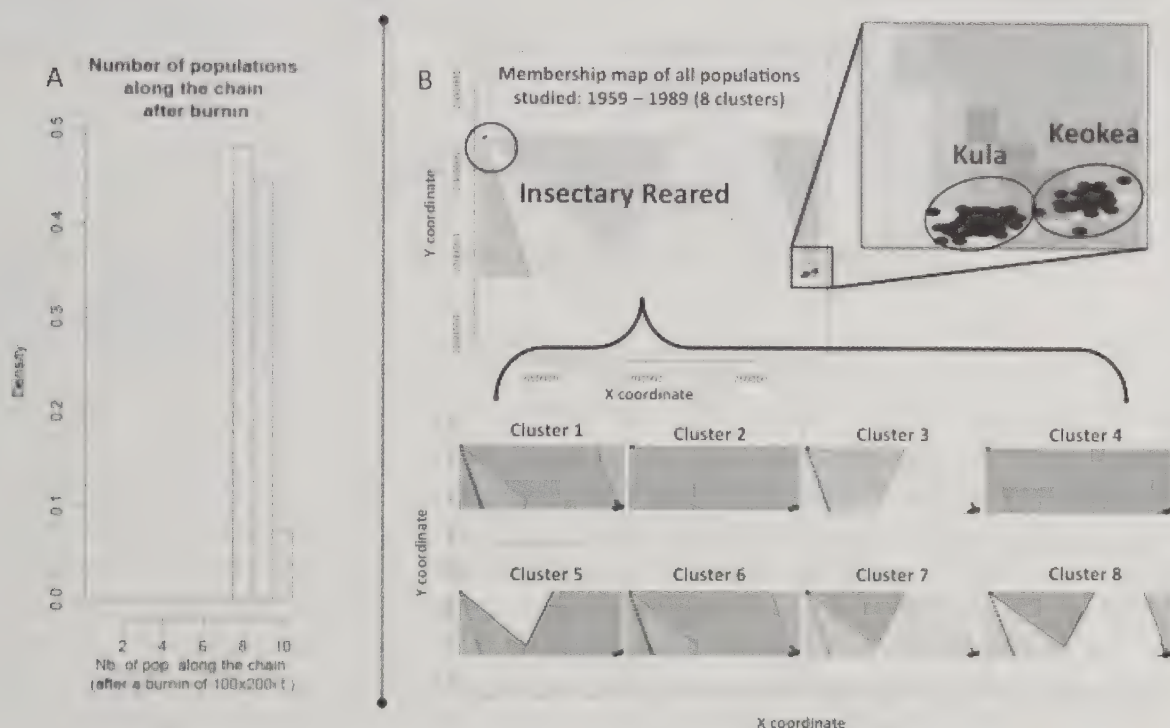


Fig. 3. Defined clusters in both number and posterior mode of membership. The number of populations **(A)** is defined by the density of the distribution of the populations in relation to the number of populations along the MCMC chain after a burn in of 100,000. The population memberships of these defined clusters **(B)** are defined by the Voronoi tessellation cluster analyses that are then combined to form the membership map. The emphasized area of the membership map illustrates the clusters found in the areas of interest (Keokea and Kula, Maui).

Given the distribution of the data over a large geographic space, it was imperative that the Kula and Keokea areas be resolved in greater detail. Two analyses were completed for Maui populations. The first analysis included the 1959 Kula population along with the 1989 Kula and Keokea populations (Fig 4A). The second only included the 1989 Kula and Keokea data in order to better visualize the released IR population drift and genetic separation of the wild types (Fig 4B). Three clusters were defined with the wild type 1959 Kula population individuals forming a 3rd cluster (White) that has closely related individuals in both the 1989 data from Kula and Keokea (Fig. 5A). Fig. 4B shows IR population drift (Green) into Keokea (White)

as well as reduced wild type make-up of the Kula area population. The cluster tessellations in Fig. 4A seem to indicate Kula (area of IR release) admixture and migration into the Keokea region ($F_{ST} = 0.197$). The inbreeding coefficients for each population reveal moderately inbred populations in the Keokea region whereas an admixed population was present in the Kula area ($F_{IS} = 0.21$ Kula and 0.42 Keokea), as would be expected in light of the IR release in the Kula region.

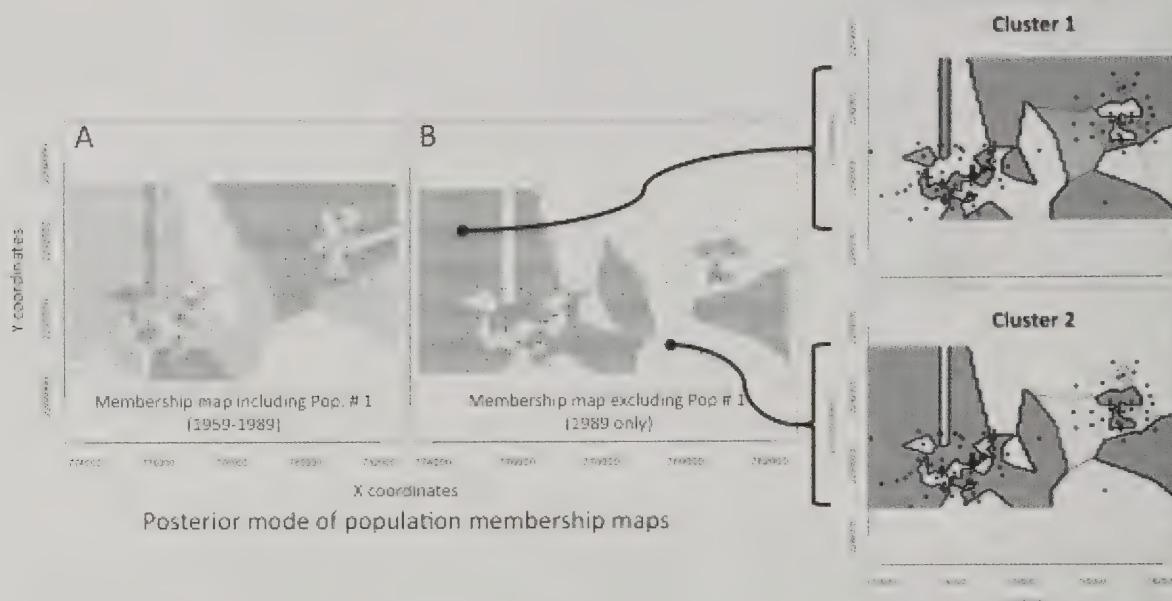


Fig 4. Posterior mode of population membership for Maui Data from 1959 to 1989. Initial analysis of all Maui population (**A**) was indicative of membership of Keokea populations with the Kula wild type and the Kula 1959 data. The three clusters shown correspond to the two theorized wild type populations from Kula and Keokea and the major IR population. Population membership with only 1989 Kula and Keokea data (**B**) indicate two major clusters between Keokea and Kula corresponding to the region of the release and control. Wild type population membership can be seen in white. Tessellation analysis shows introgression of the populations (in yellow).

DISCUSSION.

A parallel to the quality control of classically released BC agents, in which the utility and necessity of post introduction surveys are emphasized (Van Driesche & Bellows 1993; Kauffman & Nechols 1990), is the quality of augmentative release agents post introduction as compared to the natural populations (van Lenteren, 2003). If judged through a population genetics approach, quality of augmentative agents would be assessed on how differentiated those IR agents are from the natural population in the region of interest. If differentiation exists we propose that dilution and introgression of the wild haplotype would occur during inundative releases, what we coin here as a *Reverse Bottleneck*. In this case, the number of inundatively released agents would directly correlate to how dilute the wild haplotype would likely become and the likelihood of haplotype extinction per coalescent theory predictions (Avisé 2004). We suggest that the system created by Wong *et al.* (1991) is an example of this dilution process, based on the preliminary analysis and data we present here.

We hypothesized that individuals from the 1989 Kula collections would be similar to the IR population, which correlates to the probable genetic dilution of the wild haplotype. The 1959 Kula population was used to define the wild haplotype, with comparison to the captive bred individuals, to understand relationships of the 1989 Kula and Keokea collections. Fig. 2 illustrates this relationship, with an IR block of highly admixed individuals that showed equal membership to all populations, most of the 1986 IR individuals fell within this large block, as well as many individuals from 1989 Kula region and some from the Keokea region. This is in the presence of theorized wild type individuals from Kula and Keokea area (Fig. 2). Using the 1959 individuals as the learning type (pre-defined genetic structure) for an ideal “Kula wild type individual” we found individuals from the 1989 Kula samples that fell within, or had different degrees of that membership (Beaumont *et al.* 2001). We suggest that this is indicative of wild-type structural presence as well as population introgression. A second wild-type cluster was found that comprised individuals from both the Kula and Keokea regions that was independent of the IR haplotype. The presence of this haplotype structure in combination with those individuals found related to the 1959 Kula population indicates wild type presence within the regions (both pre- and post-Augmentation effort) and probable introgression of the wild type structure.

By defining the geographic origin (as seen in Figs. 1, 3 and 4) of each individual we were better able to visualize and define structural differences between regions and clusters of interest (Guillot *et al.* 2008). The initial population membership analysis (Fig. 3B) in Geneland (Guillot *et al.* 2005) denoted clustering of individuals in Kula and Keokea with the IR population as well as (as in Fig. 2) unique memberships independent of the IR haplotype. Resolving these data to a greater degree revealed relationships of wild type populations between Kula and Keokea (Fig 4A) and seems to illustrate the dominance due to dilution of the IR haplotype within the release area (Fig 4B). The inbreeding coefficient (F_{IS}) of the two populations also suggested moderate inbreeding in the Keokea region whereas the Kula region showed very low inbreeding, indicative of population dilution (Avisé 2004)

CONCLUSIONS.

Although the analysis is preliminary and meant to be suggestive, we believe that it is representative of the process which we coin a *Reverse Bottleneck*, and that the system exemplifies most inundative augmentative BC measures implemented. We define a *Reverse Bottleneck* in this paper as any instance in which a population's genetic structure is diluted in the presence of a more numerous, “non-native”, population of differing genetic structure, thereby causing an overall shift in population structure towards the more numerous haplotype. Subsequent analysis, involving a greater number of microsatellite loci, the possible inclusion of more populations and the utility of hypothesis testing techniques is planned to better resolve and understand this system. Though the current practice of the augmentative system is valid, useful and valuable, better understanding of the population and landscape genetic processes associated with this approach may improve implementation. We hope that through future analysis of this and other systems a more predictable theory describing this dilution process can be developed and directly applied.

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THE HALF-LIFE IN MOLECULAR GUT-CONTENT ANALYSIS: MODELS, ASSUMPTIONS, AND PROTOCOLS, WITH DATA FROM A ONE-PREY, MULTI-PREDATOR SYSTEM

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ABSTRACT.

Detectability half-lives weight positive assays for diagnostic prey molecules in different predator species feeding on the same prey species. We demonstrate the utility of half-lives with molecular gut-content assay data on field-collected predators of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), a system for which we have determined the half-lives of all key predators. This predator complex, which includes immatures and adults of four species in two insect orders and three families, displays order-of-magnitude variation in half-life for the Cytochrome Oxidase I sequence of a single prey egg, from 7.0 h in larval *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae) to 84.4 h in nymphal *Perillus bioculatus* (Fab.) (Hemiptera: Pentatomidae). The raw incidence of CPB DNA in the guts of 351 predators collected from conventionally tilled potato fields ranged from 11% in *C. maculata* adults to 95% in *P. bioculatus* nymphs, ranking the members of the complex thus: *C. maculata* adults < *Lebia grandis* Hentz (Coleoptera: Carabidae) adults < *Podisus maculiventris* Say (Hemiptera: Pentatomidae) adults < *P. maculiventris* nymphs < *P. bioculatus* adults < *P. bioculatus* nymphs. When adjusted for differences in half-life, the species rankings change as follows: *C. maculata* adults < *P. bioculatus* nymphs < *P. bioculatus* adults < *P. maculiventris* nymphs < *L. grandis* adults < *P. maculiventris* adults. These changes, especially elevation of *L. grandis* from next-to-worst to next-to-best candidate, and replacement of *P. bioculatus* by *P. maculiventris* as best candidate, demonstrate the value of half-life-adjusted gut-content data for evaluating predator effectiveness. Half-lives should be derived from a binary model of declining detectability. We discuss this and other technical issues in half-life determination.

UNRAVELING THE NUTRITIONAL ECOLOGY OF AN OMNIVOROUS COCCINELLID USING MOLECULAR TECHNIQUES

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ABSTRACT.

Omnivory in natural enemies is a key consideration in developing biological control programs, but the physiology that underlies digestion of foods of varying quality is poorly understood. Quantitative polymerase chain reaction (qPCR) provides a powerful tool to understand both the digestive ecology of natural enemies, as well as some of the interpretive pitfalls currently facing genetic gut content analysis (GCA). Using qPCR and marker degradation techniques, we reveal that the ability of *Coleomegilla maculata* to digest prey and non-prey foods changes over the larval stage. The changes in digestive capacity likely correspond to the relative importance of prey and non-prey foods to different larval stages. We also apply qPCR to elucidate in lab experiments the effects of prey quantity consumed, time, subsequent diet, and sample fixation and preservation on the quantity of a target prey's DNA detectable in the predator. Target prey DNA degrades differently depending on the subsequent diet of the predator, but the quantity detected is correlated with the initial amount of prey consumed. Post-feeding predator fixative protocols are of critical importance in proper use of genetic gut content analysis, with samples frozen at -20° C in pre-chilled 70% ethanol yielding the highest levels (22.8% of initial quantity) of detectable prey DNA. Results of these assays point to the value and to the complexities for applying genetic GCA to field predation studies, and also reveal new applications of this technology for understanding the nutritional ecology of omnivorous predators under controlled conditions.

INTRODUCTION.

Understanding the physiological requirements of beneficial insects is critical to successfully managing these species as agents for biological control. Most natural enemies rely on non-prey foods during at least part of their lives, and providing non-prey nutritional resources within farmland often promotes the abundance of predators and parasitoids while reducing pest populations (Lundgren 2009; Wäckers *et al.* 2005). Ontogenetic changes in the nutritional needs of an insect influence their fitness and behavior, and a better understanding of when prey and non-prey foods are of greatest importance to a beneficial omnivore may help target conservation practices that promote their contribution to biological control.

Coleomegilla maculata DeGeer (Coleoptera: Coccinellidae) is a New World lady beetle common to cropland where it acts as a biological control agent. It is highly polyphagous; in addition to being an important source of mortality to pests like aphids, and lepidopteran and coleopteran eggs (Weber *et al.* 2009), *C. maculata* frequently consumes non-prey foods such as vegetation, pollen and fungal spores

(Lundgren *et al.* 2005; Lundgren *et al.* 2004; Moser *et al.* 2008). For these reasons, *C. maculata* has become a model organism for examining the physiological and ecological implications of omnivory for biological control (Lundgren & Wiedenmann 2004; Michaud & Grant 2005; Moser *et al.* 2008).

Genetic gut content analysis (GCA) is an indirect but powerful tool for identifying which natural enemies consume a target pest under field conditions. Traditional PCR-based detection systems rely on frequency of predation as their experimental endpoint. The recent application of quantitative PCR (qPCR) to predation ecology has allowed the quantitation of prey DNA detectable within a predator as an additional more sensitive measure of predation. However, numerous properties inherent in GCA challenge the interpretation of field-obtained results.

Using *C. maculata* as a model system, we conducted a series of laboratory experiments aimed at 1) determining some of the methodological influences of both the frequency of detection and amount of DNA detectable within a predator's gut, and 2) applying DNA marker degradation rates (as measured by GCA) to determine how the feeding ecology of this omnivorous insect changes over its larval life.

MATERIALS & METHODS.

Study organisms.

Coleomegilla maculata were field-collected in Beltsville, MD, USA, and were maintained in culture for less than 6 mth. Colorado potato beetle (*Leptinotarsa decemlineata* Say, Coleoptera: Chrysomelidae) and potato aphid (*Macrosiphum euphorbiae* (Thomas), Hemiptera: Aphididae) were collected from Beltsville and were reared on potato plants. Soybean aphids (*Aphis glycines* Matsumura, Hemiptera: Aphididae) were collected in Brookings, SD, USA, and maintained on soybean plants. Field corn, *Zea mays* L. (Hybrid 4242, Northrup King Company, Golden Valley, MN, USA), was raised in pots in the greenhouse; pollen was collected from tassels and sifted according to methods described in Lundgren & Wiedenmann (2002).

Factors influencing the outcome of qPCR GCA.

Under controlled conditions, we experimentally investigated the effects of prey quantity, time, subsequent diet, and preservation techniques on the frequency of *L. decemlineata* DNA detection and amount of *L. decemlineata* DNA detectable within *C. maculata* stomachs. More details on these experiments can be found in Weber & Lundgren (in press).

Prey quantity and time. *C. maculata* fourth instars (n = 11-17 per treatment) were fed 1, 3, or 5 *L. decemlineata* eggs (3 d old). After they consumed their meal, each larva was randomly assigned to a time treatment; these larvae were then frozen (-20° C) in 70% EtOH at 0, 1, 2, 4, or 8 h post-feeding. These preserved specimens were then subjected to qPCR (outlined below).

Subsequent diet. *C. maculata* fourth instars were each fed a single 3-d old *L. decemlineata* egg and were then divided equally into one of three diet treatments (n

= 22 in each diet treatment): no subsequent food, excess *M. euphorbiae*, or excess *C. maculata* eggs. These larvae were exposed to their subsequent diet (or lack thereof) for 1 h. Afterward, larvae were frozen (-20° C) in 70% EtOH at 0, 1, 2, 4, or 8 h post-feeding. Preserved specimens were then subjected to qPCR.

Preservation techniques. *C. maculata* fourth instars (n = 9-11 per treatment) were each fed a single 3-d old *L. decemlineata* egg, and then immediately following cessation of feeding, subjected to one of several preservation protocols commonly used for experiments and field collections. These techniques are presented in Table 1.

Ontogenetic changes in the digestion of prey and non-prey foods. *C. maculata* 1st, 2nd, 3rd, and 4th instars were starved for 24 h. Larvae were then fed excess of *A. glycines* (prey) or *Z. mays* pollen (non-prey). Each larva was allowed to consume the food for 5 min, and were then randomly assigned a termination time. Larvae (n = 10 per kill time, per food type) were frozen (-20°C) in 70% EtOH at 0, 0.5, 1, 2, 3, 4, or 6 h post-feeding. The quantity of detectable food DNA within each larval gut was measured using qPCR (see below), and the digestion rates for the marker specific to each food during the 1st, 2nd, 3rd, and 4th instars were compared using regression analysis.

qPCR procedures. The DNA of predator larvae were extracted using DNEasy Blood and Tissue extraction kits according to manufacturers instructions (Qiagen Inc., Valencia, CA, USA). Food-specific primers were developed to amplify equivalent-length (approximately 150-200 bp) sequences of *L. decemlineata* (Greenstone *et al.* 2007), *A. glycines* (Harwood *et al.* 2007), and *Z. mays* (J. G. L. unpublished), and were screened for non-specific binding within this study system. 25- μ l reactions were conducted using 12.5 μ l of Quantitect SYBR Green qPCR Master Mix (Qiagen), 225 nm of forward and reverse primers, 1 μ l of template DNA (30-228 μ g/ml), 1.5 μ l of BSA, and 10.5 μ l PCR-grade water. PCR of food-specific DNA amplicons was conducted under the following conditions: an initial step of 95°C for 15 min followed by 45-70 cycles (depending on the primer sets) of 15 s at 94°C, 30 s at 52-56°C (depending on the primer sets), and 30 s at 72°C. A dissociation curve was run at the end of each PCR to determine the length of each PCR product to ensure its identity. qPCR produced a Ct value (the PCR cycle at which target DNA is detectable above background). The inverse of this Ct value was used as a metric to compare the frequency of detection and amount of prey DNA present in the guts under the different experimental conditions.

RESULTS.

Factors influencing the outcome of qPCR GCA.

Prey quantity and time. qPCR was useful in determining the initial meal size consumed by *C. maculata*. There was substantial variability in the amount of prey DNA detectable, even under these highly controlled conditions. A reduction in DNA of 2.3 to 78,000 times occurred within minutes of the initial meal. Nevertheless, a small quantity of the DNA marker was detectable in the predators for up to 8 hr after the initial feeding.

Subsequent diet. *C. maculata* larvae that subsequently consumed prey (either *C. maculata* eggs or *M. euphorbiae*), digested the *L. decemlineata* DNA marker faster than those starved after their initial meal, in the case of *M. euphorbiae*, significantly faster. The negative exponential models revealed that the quantitative half lives of the *L. decemlineata* DNA marker were 59, 31, and 16 min for starved, *C. maculata*-fed, and *M. euphorbia*-fed *C. maculata* larvae, respectively.

Preservation techniques. There is a rapid attenuation in the amount of detectable prey DNA within predator stomachs even when the best preservation techniques are employed. This study revealed that a high proportion of predators yield positive results when specimens are stored in 70% EtOH (either frozen or left at room temperature), or antifreeze (left at room temperature), or when they are frozen at -20 or -80°C dry (Table 1). Storing at room temperature without a preservative allows rapid deterioration of the target DNA within predator stomachs. The qPCR determinations of marker quantity show that the best preservation conditions tested in this assay (in terms of frequency of detection and the amount of prey DNA detectable) involved freezing the predator at -20° C in 70% EtOH.

Table 1. Effects of various preservation techniques on the detectability of *L. decemlineata* DNA (from eggs) within the stomachs of *C. maculata* fourth instars using qPCR (from Weber & Lundgren in press).

Treatment information			% detected ^a	Egg equivalents ^b
Insect	Preservation solution	Temp		
<i>L. decemlineata</i> egg	70% EtOH	-20°C	100	1.00 ± 0.12
Fed <i>C. maculata</i>	70% EtOH	-20°C	100	0.23 ± 0.06
Fed <i>C. maculata</i>	Dry	-80°C	100	0.06 ± 0.02
Fed <i>C. maculata</i>	Dry	-20°C	90	0.03 ± 0.005
Fed <i>C. maculata</i>	70% EtOH	Room temp	80	0.008 ± 0.002
Fed <i>C. maculata</i>	Antifreeze	Room temp	90	0.007 ± 0.001
Fed <i>C. maculata</i> killed with CO ₂ ^c	Dry; 4h	Room temp	20	0.001 ± 0.001
Fed <i>C. maculata</i> killed with CO ₂ ^c	Dry; 5d	Room temp	0	0
Unfed <i>C. maculata</i>	70% EtOH	-20°C	0	0

^a % of samples in which *L. decemlineata* DNA was detectable.

^b The amount of *L. decemlineata* DNA detectable, presented as the proportion of initial meal size. Mean ± SEM.

^c After the designated duration, the sample was transferred to 70% EtOH and kept at 4°C until processing.

Ontogenetic changes in the digestion of prey and non-prey foods. Larvae of *C. maculata* became more efficient at digesting pollen as they aged. Two-factor ANOVA of the genetic GCA showed that *C. maculata* digested pollen significantly more quickly during the 4th stadium than during preceding stadia; also noteworthy is that the 4th stadium is the only one in which time had a significant effect on the quantity of pollen DNA detected (instar: $F_{3, 271} = 3.24$, $P = 0.02$; time: $F_{6, 271} = 1.38$, $P = 0.051$; interaction: $F_{18, 271} = 1.01$, $P = 0.45$). *Aphis glycines* was digested at a similar rate by all four instars of *C. maculata* (2-factor ANOVA: instar: $F_{3, 277} = 0.55$, $P = 0.65$; time: $F_{6, 277} = 3.66$, $P = 0.002$; interaction: $F_{18, 277} = 0.49$, $P = 0.96$).

DISCUSSION.

This research is in line with the hypothesis that *C. maculata* physiologically increases its dietary breadth as it ages; we also use controlled laboratory assays to illustrate several considerations when applying genetic GCA to predator-prey studies. It is clear from the application of qPCR that predators quickly digest most of the marker DNA found in prey, but retain a portion of marker in proportion to the initial meal size for several hours. Thus, qPCR can be used to estimate initial meal size under controlled conditions.

Sample preservation is a critical consideration in GCA, and our data suggests that freezing predators in 70% EtOH yields the best results of the tested preservation methods (although there may be room for further improvement, with other protocols). Finally, the detectability of a target DNA sequence within a predator's gut can be affected by its subsequent diet; in our experiments, consumption of alternative prey after target pest ingestion resulted in more rapid disappearance of the target marker than if the predator was subsequently starved. All of these considerations make interpreting field measures of predation using GCA challenging. At the same time, understanding the factors that alter the result of genetic GCA underscores the usefulness of this as a tool for understanding predator digestion efficiency under regimented laboratory conditions.

As they age, herbivorous insects are known to change the amount, as well as the types, of food they consume. Characteristically, insects consume more food the older they get, but they convert this food into biomass less efficiently as they age (Scriber & Slansky 1981). Moreover, herbivorous insects change their feeding position on plants as they age and self-select nutrients according to stadium-specific dietary needs (Browne 1995). While predatory insects also consume more food as they get older, little is known about how their dietary efficiency or breadth changes as they age.

Several studies suggest that omnivores (i.e., zoophytophagous heteropterans) are able to use plant-based foods during their earliest stadia, but the requirement for nutrients inherent in prey increases as an omnivore ages (Eubanks & Denno 1999; Lundgren *et al.* 2008). The current research suggests that dietary breadth is greatest during the preimaginal stages of an omnivore, a different life history strategy than that described for many omnivores. *C. maculata* consumes more corn pollen as 4th instars than as 3rd or 2nd instars (Lundgren *et al.* 2005), and this omnivore becomes more efficient at converting pollen to biomass during the final larval stadium (Lundgren & Wiedenmann 2004).

Our research uses qPCR-based GCA to show that *C. maculata* digests prey equally well throughout the larval stage, but increases its capacity to digest non-prey foods as it ages, allowing greater trophic flexibility to complete its development. This may be an adaptation to the local depletion of prey resources over time. In this scenario, the early instars consume all the available prey that a female provides to her offspring as a consequence of her ovipositional decision; subsequently, the

larvae must seek alternative resources and undergo strong selection for dietary flexibility and breadth.

The importance of when and what nutrients to provide to natural enemies, most of which are omnivorous, has numerous implications, including for mass production of biological control agents, supporting attraction and augmentation, and providing appropriate nutritional resources to conserve endemic natural enemies in cropland.

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GENETIC VARIATION IN *MICROCTONUS* SPP. (HYMENOPTERA: BRACONIDAE): MOLECULAR DATA CAN IMPROVE BIOLOGICAL CONTROL

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ABSTRACT.

The Palaearctic parasitoid *Microtonus aethiopoides* Loan and the South American parasitoid *M. hyperodae* Loan (Hymenoptera: Braconidae) have been introduced to New Zealand as biological control agents of weevil pests of pasture. Genetic variation in both species has been explored in their natural range as well as in New Zealand. In *M. aethiopoides*, genetic variation is much more strongly correlated with host taxon than with sampling location, whereas *M. hyperodae* variation, although much smaller, appears to be linked to geographical location. These genetic differences are correlated with the varying biological control outcomes following the introduction of the two *Microctonus* species and demonstrate that genetic studies of parasitoids can improve success rates and environmental safety in biological control.

KEYNOTE ADDRESS

'NEW ORGANISMS': A NEW APPROACH TO MANAGING THE DELIBERATE

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ABSTRACT.

New Zealand is an isolated archipelago of continental fragments in the South Pacific. Its native biota is unique in combining the remnants of Gondwanan species and later arrivals, all of which have passed through a filter of distance, inundation, and glaciations. However the greatest test for our biota has been the last thousand years with the arrival of the first Polynesian settlers. This first wave brought only a few foreign elements such as rats, dogs, and kumara. However the real assault began in 1769 with the discovery of New Zealand by Cook and the consequent colonisation. The next 150 years saw both the accidental and intentional introduction of a vast array of plants and animals. Many of these introductions were to develop primary industry, however many introductions were either part of the 'gardening of New Zealand' or in the case of mammals the 'gentrify' the countryside, such as the actions of Acclimatisation Societies. In 1996 the Hazardous Substances and New Organisms Act was passed which turned New Zealand's approach to new species around. In the past where there had been black lists of those things that could not enter there was now a white list of those things approved entry. All species that meet the definition of new organism are considered to be a hazard until proven otherwise through risk assessment. An important feature of the regulatory process is when the decision to approve or decline the introduction of an organism is made the beneficial effects of the organism are weighed against the adverse effects. The nature of beneficial and adverse effects to the environment, to the health and safety of people, to societies and communities, to the economy, and on Māori culture will be explored.

SESSION 5

GMO'S AND BIOLOGICAL CONTROL

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Pest resistant germplasm is an important part of the foundation of integrated pest management (IPM) programs. With recent advances in molecular biology, it has become possible to transfer genes that provide plant resistance to certain groups of pests. The first genetically modified (GM) insect-resistant maize variety expressing a Cry protein from *Bacillus thuringiensis* (Berliner) (Bacillaceae) (Bt) was released in 1996. By 2007, the area planted to Bt maize and cotton varieties reached 42.1 million hectares in 22 countries. Thus, Bt plants have become important components of maize and cotton IPM programs worldwide and other GM insect-resistant crops are expected to be commercialized in the near future (Romeis *et al.* 2008). IPM systems should rely on a diverse set of tactics to create stable management systems. Fundamental to these is the enhancement of biological control through conservation of existing natural enemies or through introduction of new ones through inoculation or inundation. Thus, it is important to minimize the non-target effects of other components of IPM such as Bt plants and insecticides. There is a considerable amount of information available on how the introduction of Bt varieties has impacted biological control organisms and their functions in different crops. In this session, examples will be provided from different regions of the world and different crops on how Bt plants may affect natural enemies directly (by exposing them to the insecticidal protein) or indirectly through changes in crop management, i.e. through the reduction in chemical insecticides. Evidence will be presented that the plant-expressed Cry proteins have a very narrow spectrum of activity and thus pose a negligible risk to predators and parasitoids. Furthermore, in crop systems where the deployment of Bt varieties has led to a decline in insecticide use, biological control organisms have benefited significantly. Consequently, like traditionally bred insect-resistant crops, Bt crops have shown to be effective components of IPM programs and helped to strengthen biological control.

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COMPATIBILITY OF BIOLOGICAL CONTROL WITH BT MAIZE EXPRESSING Cry3Bb1 IN CONTROLLING CORN ROOTWORMS

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ABSTRACT.

The western corn rootworm, *Diabrotica virgifera virgifera*, is one of the most devastating maize pests in the USA and is currently invading Europe. Genetically engineered maize expressing the Cry3Bb1 protein derived from *Bacillus thuringiensis* Berliner (Bt) is a very promising control option and has already been commercialized in the USA. One crucial part of the environmental safety assessment of transgenic insect-resistant crops is the evaluation of potential risks to non-target species with a focus on organisms providing ecosystem services, like decomposition, pollination and biological control. This paper gives an overview of published studies on effects of Cry3Bb1-expressing Bt maize on non-target invertebrates. Most studies conducted in the laboratory or glasshouse demonstrate no effects of Cry3Bb1-expressing Bt maize or purified Cry3Bb1 protein on non-target species. In the field no detrimental impact of rootworm-resistant Bt maize was reported. In contrast, chemical insecticides were found to affect invertebrates when applied to plant foliage and occasionally also when used as soil insecticides. Seed coating did not seem to influence non-target invertebrate species. A risk assessment for a generalist predator, the spider *Theridion impressum* was conducted. The spider was found to be exposed to Bt protein after analyzing its prey spectrum and the Cry3Bb1 concentration in potential prey species. As feeding studies with Bt maize fed prey and pollen did not indicate adverse effects, we conclude that Bt maize poses a negligible risk for the spider. Finally, the interaction of an entomopathogenic fungus, *Metarhizium anisopliae*, with Bt maize and *D. v. virgifera* has been studied in the laboratory. While feeding on Bt maize delayed development of *D. v. virgifera* larvae, there was no difference in infection rates between Bt and control maize treatments. Our work and the published literature indicate that Cry3Bb1-expressing Bt maize is compatible with biological control for sustainable control of *D. v. virgifera*.

INTRODUCTION.

Corn rootworms, *Diabrotica* spp. (Coleoptera: Chrysomelidae), are the most devastating maize pest complex in the USA (Hellmich *et al.* 2008; Ward *et al.* 2005) and the Western corn rootworm, *Diabrotica virgifera virgifera* LeConte, was recently introduced to Europe where populations are spreading rapidly (Kiss *et al.* 2005). When maize plants are infected by larvae, they suffer from root feeding which may result in severe yield losses (Hellmich *et al.* 2008). Crop rotation is most effective in controlling corn rootworms, as eggs overwinter in the soil and larvae have a narrow food spectrum including maize and a few other grasses (Clark & Hibbard 2004; Oyediran *et al.* 2004). However, beetles managed to adapt to maize biannually rotated with soybean (Levine & Oloumi-Sadeghi 1991). In regions where crop

rotation is not an option, rootworm damage is usually reduced with soil insecticides against larvae and occasionally also with foliar insecticide applications targeting adults. In the USA, 60% of total insecticides used on maize are applied against *D. v. virgifera*, which is named “billion dollar bug” because of high insecticide costs and crop losses (Hellmich *et al.* 2008; Ward *et al.* 2005;). However, broad spectrum insecticides are known to result in adverse effects on soil invertebrates, success in reducing rootworm damage is often inconsistent and resistance to the active ingredient is usually observed within several years (Levine & Oloumi-Sadeghi 1991).

In agricultural fields, naturally occurring enemies like predators, parasitoids, and pathogens attack most herbivorous species and provide natural pest control that keeps populations of many herbivores below the economic damage level (Sunderland *et al.* 1997; Symondson *et al.* 2002). Although corn rootworm eggs, larvae and adults are likely to be consumed by a range of rather opportunistic generalist predators (Kuhlmann & van der Burgt 1998; Levine & Oloumi-Sadeghi 1991; Toth *et al.* 2002), no effective specialist natural enemies were found to attack *D. v. virgifera* in Central Europe (Toepfer & Kuhlmann 2004). The lack of effective specialist enemies and pathogens is typical for invasive species and one important factor for population densities building up.

One strategy to respond to this lack of specialists is to mass rear and release natural enemies to suppress the pest. Field trials with entomopathogenic nematodes have demonstrated promising reductions in root damage and effort has been made to develop suitable application techniques (Toepfer *et al.* 2008). Similarly, entomopathogenic fungi show potential for biological control as laboratory and field studies revealed that certain strains were able to kill *D. v. virgifera* larvae and beetles effectively, and found to persist in the soil (Krueger & Roberts 1997; Mulock & Chandler 2000; Pilz *et al.* 2007; Pilz 2008).

A relatively novel strategy for controlling corn rootworms is the planting of genetically engineered (GE) maize expressing Cry3Bb1 protein from the bacterium *Bacillus thuringiensis* Berliner (Bt) (Bacillales: Bacillaceae) (Vaughn *et al.* 2005). Constructs expressing Cry34/35 or modified Cry3A were also commercialized (Hellmich *et al.* 2008). In all available Bt maize varieties, the insecticidal protein is expressed constitutively in the plant and prevents root damage by larvae either due to feeding deterrence or direct toxicity. Similar to insecticidal GE crops targeting lepidopteran pests, acreages planted to rootworm-resistant maize are continuously increasing in the USA (James 2007) as growing Bt maize has the potential to reduce insecticide applications and yield losses (Demont & Tollens 2005; Hellmich *et al.* 2008). On the other hand, the continuous exposure to a high dose of a single Bt protein may lead to the development of resistance in the target pest, which requires resistance management strategies, such as the planting of conventional maize as refuges (Ferré *et al.* 2008; Hellmich *et al.* 2008).

Before new GE crops can be cultivated commercially, risks to human health and the environment have to be assessed (pre-market risk assessment) and evaluated by regulatory agencies. In addition to regulatory studies that are needed for product registration and conducted by the applicant, scientific publications are readily incorporated in the risk assessment as they provide basic data on the properties of the insecticidal compounds and on the ecology of the plants, pests, beneficial species and their interaction in agro-ecosystems. One crucial part of the

environmental safety assessment of transgenic insect-resistant crops is the evaluation of potential risks to non-target species with a focus on organisms providing ecosystem services, like decomposition, pollination and biological control (Nap *et al.* 2003; Conner *et al.* 2003). This includes the determination of exposure to the active insecticidal compound and the hazard of being exposed (Raybould 2007; Romeis *et al.* 2008a). Many beneficial species are exposed to the insecticidal protein by feeding directly on plant material (including pollen). When natural enemies consume prey which has previously fed on Bt plants, the insecticidal protein is transferred along the food chain (Romeis *et al.* 2008b). Additionally, species living below ground encounter Bt protein remaining in plant residues, residue-leachates or root exudates (Icoz & Stotzky 2008; Saxena *et al.* 2002). However, a certain species is only at risk if the transgenic protein shows toxicity at a realistic level of exposure (Raybould 2007). While estimating exposure requires knowledge of the concentration of biologically active insecticidal protein in the food (Romeis *et al.* 2008b), toxicity is tested initially on a number of surrogate species using laboratory assays (Garcia-Alonso *et al.* 2006; Romeis *et al.* 2008a).

Recently, a number of publications on the effects of rootworm-resistant maize on non-target invertebrates became available. Thus the first aim of this paper was to compile data from peer-reviewed publications and registration documents of Cry3Bb1-expressing maize. Laboratory and glasshouse experiments as well as field trials published until October 2008 were covered. In the second part, we report on a risk assessment performed for a generalist predator common in European agroecosystems including maize, the spider *Theridion impressum* L. Koch (Araneae: Theridiidae). Finally, data on the interaction of Bt maize, western corn rootworm and an entomopathogen, the fungus *Metarhizium anisopliae* (Metchn.) Sorokin (Basidiomycetes: Meripilaceae) are presented.

EFFECTS OF CRY3BB1-EXPRESSING BT MAIZE ON NON-TARGET INVERTEBRATES.

Laboratory and Glasshouse Studies.

Data from the laboratory or glasshouse are available for 7 herbivore and 15 predator species, but only for 1 parasitoid, 1 pollinator, 3 decomposer and 2 aquatic species (Table 1).

Herbivores like larvae of the Monarch butterfly, *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae), the ladybird beetle, *Epalachna vigintioctopunctata* (F.) (Coleoptera: Coccinellidae) and the leaf beetle *Galerucella vittaticollis* Baly (Coleoptera: Chrysomelidae) did not suffer when feeding on Bt maize pollen provided on leaf discs (EPA 2003; Mattila *et al.* 2005; Shirai 2006). This is interesting, as the two latter species belong to same insect order and one of them even to the same family as the targets of Cry3Bb1 expressing maize, i.e. corn rootworms. Bt maize roots were more attractive to the bulb mite *Rhizoglyphus robini* Claparede (Astigmata: Acaridae) than control maize roots in 1-day choice experiments (Carter *et al.* 2004). However, Bt maize seeds were coated with different fungicides than control seeds, which might have influenced the mites. In addition, no-choice experiments indicated similar acceptance by *R. robini* (Carter *et al.* 2004). Weight of two slug species feeding on Bt maize leaves remained similar compared to feeding on leaves of the

Table 1. Effects of Cry3Bb1-expressing maize or purified Cry3Bb1 on non-target invertebrates under confined conditions. Unless otherwise stated Bt maize was compared to the corresponding non-transformed near-isoline and diet containing Bt protein to pure diet.

Invertebrate species	Stage tested	Bt protein source	Provided food	Reported effects	Reference
Herbivores					
Lepidoptera: Nymphalidae <i>Danaus plexippus</i> (L.)	Larvae	MON863	Milkweed leaves dusted with up to 3200 pollen grains/cm ²	No effect on development time, survival, weight and feeding after 4 days exposure	EPA (2003), Mattila <i>et al.</i> (2005)
Coleoptera: Coccinellidae <i>Epilachna vigintioctopunctata</i> (F.)	Larvae	MON863	Black nightshade leaf discs dusted with up to 2000 pollen grains/cm ²	No effect on survival and development after 10 days	Shirai (2006)
Coleoptera: Chrysomelidae <i>Galerucella vittaticollis</i> Baly	Larvae	MON863	Bitter dock leaf discs dusted with up to 2000 pollen grains/cm ²	No effect on survival and development after 10 days	Shirai (2006)
Acari: Acaridae <i>Rhizoglyphus robini</i> Claparede	Adults	MON863	Root pieces	No effect on feeding behaviour in 1-day no-choice assays, Bt maize preferred to control maize in choice experiments	Carter <i>et al.</i> (2004)
Hemiptera: Aphididae <i>Rhopalosiphum maidis</i> (Fitch)		MON863	Whole plants	Decreased weight on Bt maize	Lundgren & Wiedenmann (2005)
Mollusca: Pulmonata: Arionidae <i>Arion lusitanicus</i> Mabille	Adults	MON88017	Leaves	No effect on weight and feeding after 3 days	Zurbrugg & Nentwig (<i>in press</i>)
Mollusca: Pulmonata: Limacidae <i>Deroceras reticulatum</i> (Muller)	Adults	MON88017	Leaves	No effect on weight and feeding after 3 days	Zurbrugg & Nentwig (<i>in press</i>)
Predators					
Coleoptera: Coccinellidae <i>Adalia bipunctata</i> (L.)	Larvae	Purified	Lepidopteran eggs sprayed with 0, 5, 25 and 50 µg Cry3Bb1/ml solution	Decreased survival of 1st instar at 25µg/ml, no effect at other (including higher) doses and on later instars, development time and weight	Schmidt <i>et al.</i> (<i>in press</i>)
Coleomegilla maculata (DeGeer)	Larvae	MON863	Pollen (50%) mixed with fruit fly eggs and bee pollen	No effect on development time, survival and weight	EPA (2003)
			Pollen and 1 aphid in 3rd and 4th instar	No effect on development time, survival and weight, no consequences for adult fecundity and fitness (flip time, walking speed)	Lundgren & Wiedenmann (2002)
			Pollen and 3 aphids in 2nd, 3rd and 4th instar	No effect on development time, survival, weight, no consequences for adult longevity	Ahmad <i>et al.</i> (2006a)
			Pollen (50%) mixed with fruit fly eggs	No effect on development time, survival and weight	Duan <i>et al.</i> (2002)

Table 1. Suite

<i>Hippodamia convergens</i> Gue.-Men.	Adults	MON863	Aphids reared on Bt maize (no exposure as aphids did not contain Cry3Bb1)	No effect on development time, survival, weight, fitness (flip time, walking speed) and fecundity	Lundgren & Wiedenmann (2005) Duan <i>et al.</i> (2002), EPA (2003) EPA (2003) EPA (2003)
	Adults	Purified	Pollen (50%) mixed with fruit fly eggs	No effect on survival and fecundity during 30 days	
		MON863	400 and 8000 µg Cry3Bb1/ml honey diet	No effect on survival after 10 days	
			Pollen (50%) in honey	No effect on survival after 15 days	
Coleoptera: Carabidae	Adults	MON863	Pollen (ca. 10%) mixed into or sprinkled on diet	No effect on longevity	Ahmad <i>et al.</i> (2006a) Ahmad <i>et al.</i> (2006a) Duan <i>et al.</i> (2006) Mullin <i>et al.</i> (2005) Mullin <i>et al.</i> (2005)
	Adults	MON863	Pollen (ca. 10%) sprinkled on diet	No effect on longevity, fecundity and fertility	
	Larvae	Purified	930 µg Cry3Bb1/g artificial diet	No effect on survival, development time and weight	
	Adults	MON863	Pollen	No effect on longevity	
	Adults	MON863	Pollen	No effect on longevity	
Heteroptera: Anthrenorididae	Nymphs	Purified	930 µg Cry3Bb1/g bee pollen diet encapsulated in parafilm domes	No effect on development time and survival	Duan <i>et al.</i> (2008a)
Neuroptera: Chrysopidae	Larvae	Purified	400 and 8000 µg Cry3Bb1/ml in moth egg diet	No effect on survival after 10 days	EPA (2003) Li <i>et al.</i> (2008) Li <i>et al.</i> (2008)
	Adults	MON88017	Pollen and sucrose solution	No effect on survival, pre-oviposition period, fecundity, fertility and weight during 28 days	
		Purified	150 µg Cry3Bb1/g dry weight artificial diet	No effect on survival, pre-oviposition period, fecundity, fertility and weight during 28 days	
Parasitoid	Adults	Purified	400 and 8000 µg Cry3Bb1/ml in artificial diet	No effect on survival at 400 µg/ml, 45% decreased survival at 8000 µg/ml (not significant)	EPA (2003)
Hymenoptera: Apidae	Larvae	Purified	1790 µg Cry3Bb1/ml inoculated into brood cells prior to capping	No effect on development and survival	EPA (2003)
	Adults	Purified	360 µg Cry3Bb1/ml in 30% sucrose solution	No effect on survival and behaviour	
Decomposers	Adults	MON863	Soil with growing maize plant, soil amended with ground maize tissue	No effect on survival and weight after 12-45 days	Ahmad <i>et al.</i> (2006b) EPA (2003) EPA (2003)
	Adults	Purified	167 µg Cry3Bb1/g artificial soil substrate	No effect on survival and weight after 14 days	
	Adults	MON863	0.5, 5 and 50% leaf tissue in granulated brewer's yeast	No effect on survival and reproduction	

Aquatic species				
Diptera: Chironomidae <i>Chironomus dilutus</i> Shobanov, Kiknadze & Butler	Larvae	MON863	Bt maize root extracts in artificial diet at 0, 17, 30 and 48 ng Cry3Bb1/ml	Decreased survival with higher amounts of added Bt maize root extract (no comparison with control root extracts), no effect on growth
				No effect on mortality and behaviour after 2 days
Diplostera: Daphniidae <i>Daphnia magna</i> Straus	Larvae	MON858	120 mg pollen/l water	EPA (2003)
				Transformation event of Bt maize; "purified" denotes that Bt protein produced in microbes was used

corresponding near-isoline, even though the experiment lasted only 3 days (Zurbrügg & Nentwig *in press*). Experiments with the aphid *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) revealed a reduction in body weight when developing on Bt maize (Lundgren & Wiedenmann 2005). However, phloem sap, the food aphids feed on, has been shown to transport no or only traces of Bt protein, thus aphids were not exposed and effects most likely due to different plant properties of the maize lines compared.

Work on predators focused clearly on coccinellid and carabid beetles, as they are among the most important predators in maize fields and beetles are the target group of rootworm-resistant Bt maize. The ladybirds *Coleomegilla maculata* (DeGeer) and *Hippodamia convergens* Gue.-Men. (Coleoptera: Coccinellidae) did not show susceptibility to Cry3Bb1 in a range of feeding assays with purified Cry3Bb1 and maize pollen (Ahmad *et al.* 2006a; Duan *et al.* 2002; EPA 2003; Lundgren & Wiedenmann 2002). The only tritrophic study published used *C. maculata* and aphids as prey (Lundgren & Wiedenmann 2005). No effects were observed, but the beetles did not ingest Cry3Bb1 as it was not detectable in the aphids. On the other hand, first instar *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) showed 33% mortality when provided with lepidopteran eggs that were treated with Cry3Bb solution at a concentration of 25 µg/ml (Schmidt *et al. in press*). However, the study likely suffered from methodological problems indicated by the following points: (1) the control mortality in the first larval stage was high (21%); (2) a higher concentration of 50 µg Cry3Bb/ml did not result in significant differences to the control group; (3) developmental time of surviving larvae was not affected; (4) later larval stages had a low control mortality and did not show effects at any Cry3Bb concentration; and (5) when Cry1Ab (known to be specific for Lepidoptera) was fed to the beetles, reported effects on first instar mortality were even stronger than for the Coleoptera-specific Cry3Bb. Studies with carabid beetles provided with pollen or purified Cry3Bb1 revealed no detrimental effects (Ahmad *et al.* 2006a; Duan *et al.* 2006; Mullin *et al.* 2005). Similarly, no impact of Cry3Bb1 on the non-beetle predators *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) was observed (Duan *et al.* 2008a; EPA 2003; Li *et al.* 2008).

Data on one parasitoid, *Nasonia vitripennis* (Walker) (Hymenoptera: Braconidae) and one pollinator, the honey bee *Apis mellifera* L. (Hymenoptera: Apidae) are available from regulatory risk assessment and did not reveal any effect of purified toxin at high concentrations. The community of decomposers was addressed in studies with earthworms and the collembolan *Folsomia candida* Willem (Collembola: Isotomidae). No effects were evident at Cry3Bb1 levels exceeding maximal environmental concentrations (Ahmad *et al.* 2006b; EPA 2003). The aquatic midge *Chironomus dilutus* Shobanov, Kiknadze, & Butler (Diptera: Chironomidae) showed increasing mortality when provided food amended with increasing amounts of Bt maize root extracts (Prihoda & Coats 2008). However, it remains unclear if effects were due to the presence of Cry3Bb1 or other compounds in the root extracts, as no control treatments with increasing concentrations of non-Bt maize root extracts were included. Another aquatic species, *Daphnia magna* Straus (Diplostraca: Daphniidae), did not show effects when exposed to high amounts of Bt maize pollen mixed into water (EPA 2003).

Field Studies.

The invertebrate fauna in the plant layer, on the ground and in the soil has been investigated in field trials with Cry3Bb1-expressing Bt maize (Table 2). Besides the untreated, non-transformed near isoline, most studies included alternative pest control methods such as seed coating, soil insecticides or foliar insecticides.

The abundance of plant dwelling arthropods including herbivores, predators and parasitoids, measured by visual counts, sticky traps and sweep netting, was generally reported to be similar in plots of the Bt and untreated control maize line (Ahmad *et al.* 2006a; Al-Deeb & Wilde 2003; Bhatti *et al.* 2005a; McManus *et al.* 2005; Rauschen *et al.* in press). Bhatti *et al.* (2005a) included *D. v. virgifera* beetles and another chrysomelid species in their analysis and showed, as expected, reduced numbers in Bt maize. Only one study has directly assessed the biological control function and reported no differences in predation of corn borer egg masses between Bt and control maize (Ahmad *et al.* 2006a). Seed treatments and soil insecticides had generally no influence on plant dwelling arthropods, in contrast to foliar application of permethrin, which reduced coccinellids, lacewings and nabids and resulted in increased aphid populations (Ahmad *et al.* 2006a; Al-Deeb & Wilde 2003; Bhatti *et al.* 2005a; McManus *et al.* 2005).

Species living on the ground were captured using pitfall traps. Predators, herbivores and detritivores in Bt maize showed similar activity densities when compared to the corresponding non-transformed maize plants (Ahmad *et al.* 2005; Al-Deeb & Wilde 2003; Bhatti *et al.* 2005b; Bitzer *et al.* 2005). While seed treatment did not influence ground dwelling invertebrates, the use of tefluthrin as a soil insecticide resulted in reduced numbers of carabids and spiders in one of 3 studies (Bhatti *et al.* 2005b) and increased abundance of surface active Collembola (Bitzer *et al.* 2005). Spiders were furthermore affected by foliar application of permethrin (Bhatti *et al.* 2005b).

Invertebrates living in the soil were studied by a range of methods like Tullgren, MacFadyen and pan trap extraction, bulb mite traps and centrifugal flotation. No detrimental effects of Bt maize on predators, herbivores and detritivores were reported (Ahmad *et al.* 2005; Al-Deeb *et al.* 2003; Bhatti *et al.* 2005b; Bitzer *et al.* 2005; Carter *et al.* 2004; Hönemann *et al.* 2008). Only the western corn rootworm was less abundant in soil samples from Bt maize (Bhatti *et al.* 2005b). Similar to above ground species, seed coating did not seem to influence invertebrate abundance, while one of 5 studies found soil insecticide to reduce herbivorous Nitidulidae (Coleoptera) and predatory Carabidae and Japygidae (Diplura) (Bhatti *et al.* 2005b). Furthermore, soil-dwelling Collembola were counted more frequently in soil treated with tefluthrin (Bitzer *et al.* 2005).

In conclusion, most studies conducted in the laboratory or glasshouse demonstrate that Cry3Bb1-expressing Bt maize is specific to Chrysomelidae. No detrimental effects on non-target species including Coleoptera were reported with the exception of 2 studies on the ladybird beetle *A. bipunctata* and the midge *C. dilutus*. However, both studies suffered from serious methodological problems that shed some doubts whether the reported effects were caused by the ingestion of Cry3Bb1.

Table 2. Effects of Cry3Bb1-expressing maize and insecticide applications on non-target invertebrates under field conditions. Unless otherwise stated Bt maize and insecticide treatments are compared with untreated near-isolines.

Transform. event	Sampling method	Sampled taxa	Reported effects	Reference
Plant-dwelling arthropods				
MON863	Visual counts; predation of moth eggs	Predators (Het: Anthocoridae, <i>Orius insidiosus</i> ; Col: Coccinellidae, <i>Coleomegilla maculata</i> , <i>Hippodamia convergens</i> , <i>Scymnus</i> spp.); activity of chewing & sucking predators	No effect of Bt maize, seed coat (clothianidin) or soil insecticide (tefluthrin, bifenthrin, pyrethroid) on abundance and egg predation	Ahmad <i>et al.</i> (2006a)
MON863	Sticky traps	Predator (Col: Coccinellidae, <i>C. maculata</i>)	No effect or slightly increased abundance in Bt maize compared to near-isoline (both with seed coat imidacloprid), no effect of soil insecticide (tefluthrin, pyrethroid)	McManus <i>et al.</i> (2005)
MON863	Visual counts	Predators (Het: Anthocoridae, <i>O. insidiosus</i> ; Col: Coccinellidae, <i>C. maculata</i> , <i>H. convergens</i> , <i>Scymnus</i> spp.)	No effect of Bt maize, seed coat (imidacloprid or undefined) or soil insecticide (chlorpyrifos, terbufos, tefluthrin, carbofuran, tebufiprimofos & cyfluthrin)	Al-Deeb & Wilde (2003)
MON88017	Sweep netting	Herbivore (Hem: Miridae, <i>Trigonotylus caelestialium</i>)	No effect of Bt maize, but differences among conventional varieties	Rauschen <i>et al.</i> (<i>in press</i>)
MON863	Sticky traps	Herbivores (Col: Chrysomelidae; Hem: Aphididae); Predators (Col: Coccinellidae; Hem: Anthocoridae; Nabidae; Neu: Chrysopidae; Dip: Syrphidae; Araneae); Parasitoids (Hym: Braconidae)	Decreased abundance of Chrysomelidae (target group) in Bt maize, no effect of Bt maize on other taxa; abundance decreased for Coccinellidae, Chrysopidae and Nabidae and increased for aphids after application of foliar insecticide (permethrin), no effect of seed coat (imidacloprid) or soil insecticide (tefluthrin)	Bhatti <i>et al.</i> (2005a)
Ground-dwelling arthropods				
MON863	Pitfall traps	Herbivores (Col: Elateridae, Orthoptera: Gryllidae); Predators (Col: Carabidae, Staphylinidae; Araneae); Detritivores (Hym: Formicidae)	No effect of Bt maize, seed coat (clothianidin, neonicotinoid) or soil insecticide (tefluthrin, pyrethroid)	Ahmad <i>et al.</i> (2005)
MON863	Pitfall traps	Herbivores (Col: Elateridae, Scarabaeidae; Orthoptera: Gryllidae); Predators (Col: Carabidae, Cicindelidae, Cleridae, Staphylinidae; Araneae); Detritivores (Hym: Formicidae; Collembola)	No effect of Bt maize, seed coat (imidacloprid or undefined) or soil insecticide (chlorpyrifos, terbufos, tefluthrin, carbofuran, tebufiprimofos & cyfluthrin)	Al-Deeb & Wilde (2003)
MON863	Pitfall traps	Herbivores (Col: Nitidulidae, Scarabaeidae; Orthoptera: Gryllidae); Predators (Col: Carabidae; Staphylinidae; Araneae; Chilopoda); Detritivores (Hym: Formicidae)	No effects of Bt maize or seed coat (imidacloprid), decreased abundance of Araneae after application of soil insecticide (tefluthrin) or foliar insecticide (permethrin) and of Carabidae after application of soil insecticide	Bhatti <i>et al.</i> (2005b)
MON863	Pitfall traps	Detritivores (Collembola)	No effect of Bt maize or seed coat (imidacloprid), increased abundance after application of soil insecticide (tefluthrin)	Bitzer <i>et al.</i> (2005)
Soil fauna				
MON863	Tullgren extraction	Detritivores (Acari, Collembola)	No effect of Bt maize, seed coat (clothianidin, neonicotinoid) or soil insecticide (tefluthrin, pyrethroid)	Ahmad <i>et al.</i> (2005)

MON863	Pan trap extraction	Herbivores (Col: Chrysomelidae, Nitidulidae), Predators (Col: Carabidae; Staphylinidae; Araneae; Chilopoda), Detritivores (Diplura, Japygidae; Hym: Formicidae)	Decreased abundance of Chrysomelidae (taget group) in Bt maize, no effect of Bt maize on other taxa; decreased abundance for Carabidae, Nitidulidae and Japygidae after application of soil insecticide (tefluthrin), no effect of seed coat (imidacloprid)	Bhatti <i>et al.</i> (2005b)
MON853 MON862 MON863	Tullgren extraction Centrifugal flotation	Detritivores (Acari, Collembola) Nematodes (<i>Helicotylenchus</i> , <i>Pratylenchus</i> , <i>Tylenchorhynchus</i> , <i>Hoplolaimus</i>)	No effect of Bt maize, seed coat (imidacloprid or undefined) or soil insecticide (chlorpyrifos, terbufos, tefluthrin, carbofuran, tebufipirimfos & cyfluthrin)	Al Deeb <i>et al.</i> (2003)
MON88017	MacFadyen extraction	Detritivores (Collembola, Acari, Clitellata)	No effect of Bt maize in litter bags, no differences among conventional varieties	Hönemann <i>et al.</i> (2008)
MON863	Bulb mite trap	Herbivore (Acari: Acaridae, <i>Rhizoglyphus robini</i>)	No effect of seed coat (imidacloprid) or soil insecticide (tefluthrin), no effect of Bt maize with seed coat	Carter <i>et al.</i> (2004)
MON863	Tullgren extraction	Detritivores (Collembola)	No effect of Bt maize or seed coat (imidacloprid), increased abundance after application of soil insecticide (tefluthrin)	Bitzer <i>et al.</i> (2005)

*Insect orders are abbreviated as Col (Coleoptera), Hem (Hemiptera), Het (Heteroptera), Hym (Hymenoptera), Neu (Neuroptera)

In the field no detrimental impact of Bt maize on non-target species was reported. In contrast, chemical insecticides used to control corn rootworms were found to affect invertebrates when applied to plant foliage and occasionally also when used as soil insecticides.

RISK ASSESSMENT FOR THERIDION IMPRESSUM.

With respect to biological control, work conducted on Cry3Bb1-expressing maize clearly focused on coleopteran predators in the families of Coccinellidae and Carabidae as Cry3Bb1 targets Coleoptera. One group that has generally been neglected in risk assessment research of Bt crops are spiders, which are abundant in maize agroecosystems and considered as beneficial for pest suppression (Sunderland 1999). However, only one laboratory hazard study (Ludy & Lang 2006) with *Araneus diadematus* Clerck (Araneae: Araneidae) has been published. Consequently, we conducted a risk assessment of rootworm-resistant maize for *Theridion impressum* L.Koch (Araneae: Theridiidae), a common European spider. Analysis of the prey spectrum and the Cry3Bb1 concentration in a range of potential prey species revealed that the spider is exposed to Bt protein. However, exposure is highly variable, as the prey spectrum includes species with little or no Bt protein (e.g., aphids) as well as species with high Cry3Bb1 concentrations (e.g., mirid bugs). In feeding studies in the laboratory, field collected adult spiders were fed with Bt maize-fed prey (alternately rootworm adults and lacewings) for almost 2 months. No effects on mortality and offspring production were observed. Similarly, juveniles provided Bt maize fed prey or pollen did not indicate adverse effects on weight increase and mortality. From those results, we conclude that Bt maize poses a negligible risk for the spider.

INTERACTION OF BT MAIZE, DIABORTICA VIRGIFERA VIRGIFERA AND AN ENTOMOPATHOGENIC FUNGUS.

While a number of studies on invertebrates is available (Tables 1&2), data on the interaction of Bt crops with microbial biological control agents is scarce, even though entomopathogenic fungi, bacteria or viruses may contribute considerably to biological control and may be important for resistance management. Lawo *et al.* (2008) and Johnson *et al.* (1997a, b) worked on the interaction of entomopathogenic fungi, a lepidopteran pest and experimental genetically engineered chickpea or tobacco expressing Cry2Aa or Cry1Ac, respectively. We conducted a laboratory study on Cry3Bb1-expressing maize, *D. v. virgifera* larvae and the entomopathogenic fungus *Metarhizium anisopliae*. Second instars feeding on Bt maize developed slower, but no effects on mortality were evident. Infection rates were depending on the concentration of spore suspensions into which rootworm larvae were dipped, but no differences between Bt and control maize treatments could be observed. Adults were not influenced by Cry3Bb1 in their food. Susceptibility of adults to the fungus was generally higher than for larvae when dipped into spore suspensions, but no difference between Bt and control maize was evident. This indicates that rootworm-resistant Bt maize is compatible with biological control by entomopathogenic fungi.

CONCLUSIONS.

Our work together with the published laboratory and field studies revealed generally no detrimental effects of Cry3Bb1-expressing, rootworm-resistant Bt maize on non-target species. Similar results for other crops, mainly maize and cotton expressing Lepidoptera-active Cry proteins, were reported in recent reviews on above ground predators and parasitoids (Romeis *et al.* 2006), bees (Duan *et al.* 2008b; Malone & Burgess 2008) and below-ground species (Icoz & Stotzky 2008). Similar to other commercialized Bt crops, rootworm-resistant Bt maize contributes to sustainable agriculture by maintaining ecological services including biological control provided by spiders and entomopathogenic fungi.

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INTEGRATED PEST MANAGEMENT AND THE EFFECTS OF TRANSGENIC COTTON ON INSECT COMMUNITIES IN AUSTRALIA: LESSONS FROM THE PAST AND FUTURE DIRECTIONS

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ABSTRACT.

Transgenic Bt cotton has impacted on the Australian cotton industry by largely controlling Lepidopteran pests and contributing to a major reduction in insecticide applications. As a consequence it has strongly supported the development of IPM in Australia. Nevertheless, because of the dominance of Bt cotton, it is important to ascertain what changes have occurred in the invertebrate community because any change may require adjustments to crop management that could impact on the greater community. In our studies of Australian transgenic crops we have found no consistent difference in the diversity or species richness of the beneficial communities in unsprayed Bt and conventional crops, and crop type accounted for about 5% of the variance between these communities. The most consistent differences were higher numbers in conventional crops of Lepidoptera (as expected), Chloropidae and *Nabis kinbergii*. Some initial findings also indicated that the application of broad-spectrum insecticides in Bt crops (used to control sucking pests) can sometimes flare mites more quickly in Bt than conventional cotton. Therefore, our conclusion is that the positive effect of insecticidal transgenic cotton reducing insecticide applications overshadows the slight differences between transgenic and conventional communities. We discuss the relationship between transgenic cotton and IPM in Australia, and future directions that these technologies may take.

INTRODUCTION.

Transgenic *Bacillus thuringiensis* Berliner (Bacillaceae) (Bt) cotton has impacted on the Australian cotton industry by largely controlling Lepidopteran pests and contributing to a major reduction in insecticide applications (Fitt *et al. in press*). As a consequence, it has strongly supported the development of IPM in Australia.

The basic premise of integrated pest management (IPM) is that more than one method is used to control pests and that pests only warrant control once their numbers have reached an economically justified threshold. IPM can then develop in two directions: The first direction is a shift in the techniques used to both reduce pest numbers and then keep pest numbers low; the second direction is a shift in the level of engagement or scale at which IPM integrates with the ecosystem. Kogan (1998) identified 3 levels: (1) integration of methods at the species/population level, (2) integration of impacts of multiple pest categories (insects, pathogens, and weeds) which occur at the community level; and (3) integration of multiple pest impacts within

the total cropping system (ecosystem level). IPM can also be expanded further. Lewis *et al.* (1997) argued that IPM develops as the emphasis moves away from a “treat the symptoms” approach, to a more holistic approach which asks “why is the pest a pest?”. Lewis *et al.* (1997), like Kogan, advocate that ultimately, IPM should be undertaken within the context of the whole ecosystem.

Many advocates recognize that the strength of IPM is that it encompasses a range of techniques and levels of engagement, and that it changes in response to changes in the agricultural system in which it is based (Fitt *et al. in press*, Naranjo *et al.* 2008). IPM in Australian cotton uses a diverse variety of control methods, and manages pests at all three levels described above, depending on local conditions.

Our objective is to examine the interaction between IPM and transgenic Bt cotton in Australia. We outline the history of IPM and Bt cotton, discuss the influence of transgenic cotton on the invertebrate community, and the implications of this for IPM. We also discuss transgenic crops currently in development, their possible effect on IPM, and the future direction of IPM in Australian cotton.

THE HISTORY OF IPM AND BT COTTON.

Integrated Pest Management has a long history in Australia. Geier (1966) developed this concept (calling it “pest management”) in response to the problems caused by the use of organosynthetic insecticides (such as DDT) in the 1960s (e.g., Carson 1962). These insecticides were used to control Lepidopteran species such as *Helicoverpa* spp. (Lepidoptera: Noctuidae) which are key pests of cotton worldwide and capable of dramatically reducing cotton yield (Luttrell *et al.* 1994). Some *Helicoverpa*, including *H. armigera* (Hübner), developed resistance to insecticides quickly, rendering them ineffective within a few years of their release. For example, 34 applications of insecticides were applied in one season to one field of cotton to control *H. armigera* (Hearn 1975). Such insecticide useage decimated the beneficial populations, and often led to pest resurgence and outbreaks of secondary pests in the heavily sprayed crops (Wilson *et al.* 1998). In addition, the off-farm movement of pesticides had the potential to contaminate the surrounding environment.

According to Geier (1966), the aim of pest management is to make sure that control operations fit well with the biology of the pests; and the term “pest management” implies acceptance of the continued existence of potentially harmful species, albeit at tolerable levels of abundance. Unfortunately, after Geier wrote his paper, new insecticides including endosulfan and pyrethroid were developed and again the philosophy of pest control was to annihilate all pests (Gutierrez 1995).

However, by the early 1980s there were already signs of *H. armigera* developing resistance to both chemical groups (Forrester *et al.* 1993). To halt the establishment of resistance, the Insecticide Resistance Management Strategy (IRMS) was established, which aimed to restrict both the period during the season in which a chemical could be applied and the number of applications of a chemical per season. Cultivating the field at the end of the season (“pupae busting”) was also initiated to stop the overwintering of resistant individuals. At the same time an on-line decision support tool for pest management was created called SIRATAC (Hearn & Bange 2002; Room 1979). This approach encouraged regular insect sampling and

tried to restrict spraying by including a pest threshold for insecticides which took into consideration plant compensation and the crop development stage (Forrester *et al.* 1993). Because most of the insecticides were broad spectrum, there were very few beneficial species in these crops, which explains the notable omission of beneficial insects as part of the management of insect pests at this time.

Despite the attempts to delay resistance, *H. armigera* seriously threatened the cotton industry in Australia during the 1998-99 growing season. It had developed so much resistance to most conventional insecticides that each cotton field received on average, 14 insecticide applications (Wilson *et al.* 2004) which was not an economically viable situation. The crisis led to a greater adoption of IPM and the areawide management of pests (AWM) aiming to grow cotton profitably while tolerating some damage. This approach was strongly supported by the advent of Bt cotton, initially INGARD® in 1996 and then superseded by 2004 with Bollgard II®.

In contrast to the original Bt sprays, transgenic Bt cotton has provided much more consistent control of *Heliothis* / *Helicoverpa* spp. Bt cotton has the genetic code for Cry toxins of the bacterium *Bacillus thuringiensis* var *kurstaki* Berliner which are specific to Lepidoptera. Cry toxins kill insects by rupturing their gut wall, causing them effectively to die from septicemia (Samson & Gooday 1998, van Rie 2000). Bt cotton has had a major impact on cotton production wherever it has been commercially adopted by significantly reducing pesticide inputs (Benedict & Altman 2001, Fitt & Wilson 2000, Fitt *et al. in press*; Fitt 2008; Naranjo *et al.* 2008; Perlak *et al.* 2001; Qaim 2003). Consequently, Bt cotton has been a valuable tool when implementing IPM strategies in cotton worldwide (Fitt 2008; Fitt & Wilson 2000; Naranjo *et al.* 2008; Wilson *et al.* 2004).

In Australia, Bt cotton has greatly enhanced IPM because it has largely controlled *H. armigera* and *Helicoverpa punctigera* (Wallengren) (Fitt *et al.* 1994, Fitt & Wilson 2000, Whitehouse *et al.* 2005). This has dramatically reduced pesticide use in Australia. Ingard Bt cotton (which expressed the Cry1Ac protein) saw a 44% drop in insecticide use from 1995/96 to 2003/04, while Bollgard II® cotton (which expresses both Cry1Ac and Cry2Ab) saw a 82% drop from 2002/03 to 2005/06 (Fitt *et al. in press*). This has provided the cotton industry with the freedom to develop more soft options in the management of pests (Knox *et al.* 2006) and allowed a greater role for beneficial insects and spiders in the crop.

Now with Bollgard II®, cotton firmly established, the majority (about 85% in 2007) of the Australian cotton crop is now transgenic (Fitt *et al. in press*). Under these conditions the threat of resistance to Bt by *H. armigera* is strong, and the industry has a number of strategies in place to manage this risk, including the use of refuges, compulsory cultivation ("pupae busting") under Bt crops, narrow planting windows for Bt crops, removal of volunteer Bollgard II® plants, defined spray thresholds for *Helicoverpa* spp. in Bt crops, and the monitoring of resistance levels in field populations (Fitt *et al. in press*). However, the dominant position of Bt cotton within the industry means that even a small difference in the invertebrate community such as a change in species composition may influence how cotton should be managed using an IPM approach: Bt cotton only controls lepidopteran pests, changes in the abundance of other pests and beneficials may have an impact on IPM.

IMPACT OF BT COTTON ON INVERTEBRATES IN AUSTRALIA.

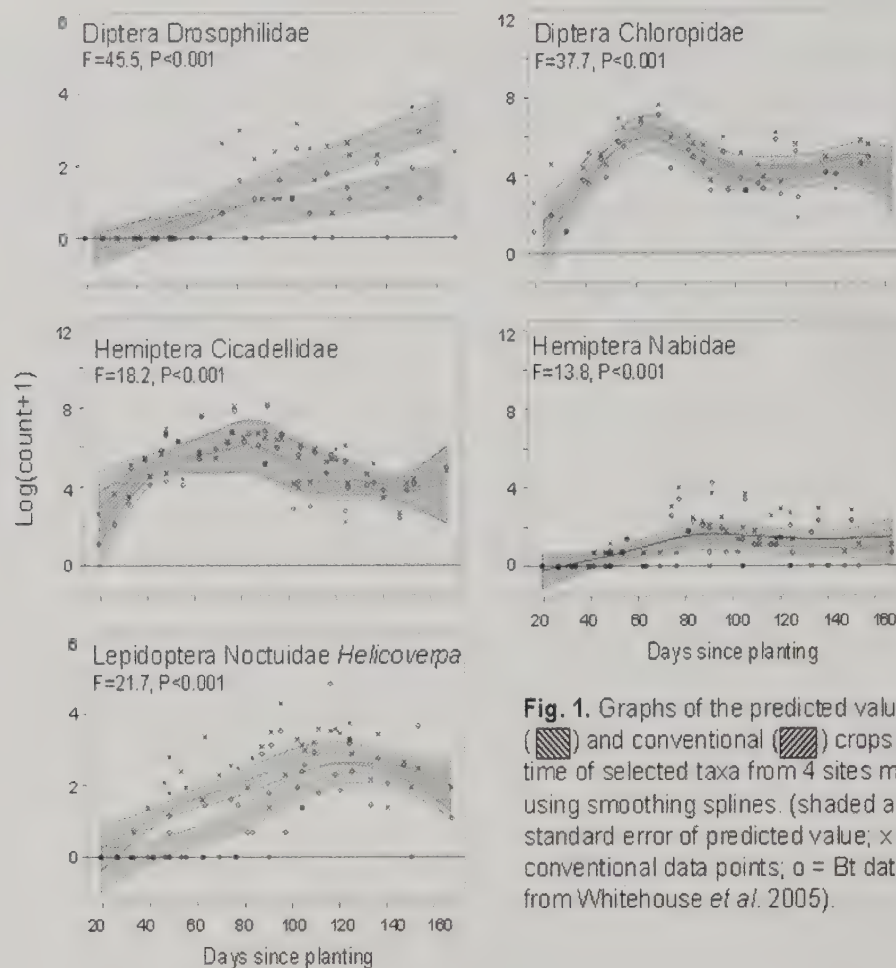


Fig. 1. Graphs of the predicted values for Bt (○) and conventional (x) crops versus time of selected taxa from 4 sites modeled using smoothing splines. (shaded areas = 1 standard error of predicted value; x = conventional data points; o = Bt data points; from Whitehouse *et al.* 2005).

Numerous studies on the direct impact of Bt cotton on the invertebrate community have shown that any changes are slight or non-existent (see Deguine *et al.* 2008; Romeis *et al.* 2006). Most studies emphasize that the reduction in insecticides associated with Bt cotton more than off-sets any slight community differences between Bt and conventional cotton (Naranjo 2005; Torres & Rubenstein 2005; Whitehouse *et al.* 2005). The situation in Australian cotton is no exception. In work that examined the impact on invertebrates of Ingard cotton compared to conventional cotton, crop type accounted for only 4.5% of the variance between the two communities (Whitehouse *et al.* 2005). Of over 100 species groups examined, the most consistent differences between unsprayed Bt and conventional communities were both higher numbers of *Helicoverpa* spp. in conventional crops (as expected); and slightly higher numbers of Chloropidae, Drosophilidae (Diptera), damsel bugs (Hemiptera, Nabidae) and jassids (Hemiptera, Cicadellidae) in conventional crops (Fig.1). The variance between Bollgard II and conventional communities was also about 5% (Whitehouse, *et al.* unpubl. Data) with Bt cotton supporting lower numbers of damsel bugs (Mansfield *et al.* 2006; Whitehouse *et al.* unpubl. Data). Although there are reports of no change in damsel bug numbers in some Bt crops such as corn (Wold *et al.* 2001) our findings agree with Naranjo (2005) who reported a

reduced number of damsel bugs in a 6 year study in Bt cotton in the United States, as did Daly & Buntin (2005) in Bt corn.

Some difference between the invertebrate communities found in unsprayed conventional and Bt cotton is to be expected given that the abundance of many lepidopteran larvae has been greatly reduced in the Bt-cotton community. This may explain why there are lower numbers of damsel bugs in Bt cotton. Damsel bugs which were fed *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) larvae that had consumed either Bt or conventional cotton showed no difference in their development, fecundity or survival (Ponsard *et al.* 2003). Thus a direct effect of Bt on damsel bugs is unlikely. Damsel bugs are generalist predators (Snyder & Ives 2003) that may attack Lepidoptera larvae and eggs (Ehler 2004), but are also predators of aphids (Elliott *et al.* 2002; Hesler *et al.* 2000, Östman & Ives 2003) and spider mites (Wilson *et al.* 1998). It may be that damsel bugs are more dependant on lepidopteran larvae than currently realized, which could partially explain their reduced abundance in Bt cotton (Whitehouse *et al.* 2005). Nevertheless, lower damsel bug numbers in Bt cotton could influence the abundances of secondary pests that they attack, such as mites.

The reduction in Chloropidae and Drosophilidae in Bt-cotton compared to conventional cotton is hard to explain, as is the role of Chloropidae in cotton. The larvae of this family are reported to feed on a range biota, including bacteria, vegetative matter (both living and rotting), and the eggs of other insects and spiders. They live beneath the skins of living frogs and as parasites of Hymenoptera (Spencer 1986). It is possible that the Chloropidae larvae were feeding on the frass from the *Helicoverpa* larvae, or the damaged plant tissue, and so are less abundant in Bt cotton which sustains less damage from lepidopteran larvae. As Chloropidae does not appear to be a pest or beneficial in cotton, its role (from an IPM perspective) is probably limited to being an alternative source of food for some predators (Whitehouse *et al.* 2005). Fewer Chloropidae in Bt cotton has no clear effect on the rest of the community.

Of concern in Bt cotton is the increase in the prominence of non target pests such as *Creotides dilutus* (Stål) (Hemiptera: Miridae). As mirids are easily controlled by most insecticides used against *Helicoverpa* spp., this difference is easily attributed to a lack of insecticides targeting *Helicoverpa* spp. In Bt cotton. In addition, mirids are attracted to healthy plants with fruit. These are more likely to be Bt crops in unsprayed conditions than conventional crops. Nevertheless, management of these pests is a concern for Bt cotton growers as it has the potential to interfere with IPM.

While Bt cotton has drastically reduced the amount of insecticide used on cotton, the rise in the prominence of mirids and other sucking pests threatens to cause some increase in insecticide use. A recent survey which documented the responses of pest managers to the presence of mirids found that a large proportion (54%) of insecticide was applied to mirids below threshold (Whitehouse 2008). In addition, 62% of the spray applications were fipronil, which can result in mite populations increasing dramatically (Wilson *et al.* 1998). The second most popular insecticide, Dimethoate, can lead to dramatic increases in whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in northern cotton growing regions. Both of these insecticides disrupt the beneficial complex. Thus unnecessarily spraying for

mirids could disrupt an IPM program by reducing the beneficial population, thereby triggering secondary pest outbreaks. The temptation to use cheap sprays (driven by the high cost of access to Bt cotton technology and low cotton prices) to control a pest which may cause damage, could chip away some of the advances IPM has made in the last decade.

Nevertheless there is evidence that pest managers are trying to combat mirids within an IPM framework. For example, to avoid disrupting IPM, pest managers have taken it upon themselves to use low rates of fipronil with the aim of conserving as many beneficials as possible (Whitehouse 2008). In addition, pest managers in whitefly sensitive regions are avoiding using dimethoate. The mirid survey showed that using mirid thresholds on commercial farms incurred no cost to yield compared with spraying at below threshold densities (Whitehouse 2008). Advertising these findings will hopefully enhance the pest manager's confidence in the mirid thresholds and reduce insecticide applications for mirids.

Another non-target pest which is more prominent in Bt than conventional cotton is mites. Again most of the increase in mites is attributed to changes in the insecticide spray regime allowing mites more opportunity to develop in Bt crops. Nevertheless there seem to be some interactions between mites and Bt cotton which are difficult to explain. Although mites accumulate Bt in their body (Dutton *et al.* 2002), there is no evidence that the presence of Bt enhances life history traits or that Bt plants are inherently better food (Whitehouse *et al.* unpubl. Data). However, mites caged with damsel bugs on conventional cotton did particularly poorly (suggesting that the mites were being eaten) but exceptionally well on Bt cotton (suggesting the mites were not being consumed; Whitehouse *et al.* unpubl. Data). Why the underlying crop type (conventional or Bt) should so strongly change the mites abundance in the presence of damsel bugs is unclear. Likewise in a study conducted over 3 seasons we found an interaction between mites, Bt cotton, and insecticides (Whitehouse, *et al.* unpubl. data): In unsprayed plots inoculated with mites there was no difference between Bollgard II® and conventional cotton in the build up of a mite population which was very slow, presumably due to an abundance of predators. However, in inoculated Bollgard II® and conventional plots that were sprayed with fipronil, the number of mites dramatically increased in both treatments, but in some experiments, the rate of increase was much greater in the Bollgard II® plots. These results suggest that while Bt cotton does not directly affect this pest, under some conditions mites do better in Bt than conventional cotton, however the mechanism is unclear.

Pupae busting and low tillage. An important part of the management of a Bt crop is to maintain efficacy. This includes ensuring that refuges are planted in which susceptible moths can develop and dilute the genetic influence of any susceptible, or partly susceptible moths developing in the Bt crop (Gould 1998). Pupae busting at the end of the season under a Bt crop is part of this management strategy: by pupae busting, any larvae that developed in a Bt crop will not be able to overwinter as pupae (diapausing) and initiate the next seasons infestations. Pupae busting to stop the overwintering of moths potentially carrying a resistant gene, was found to be a very effective tool in managing resistance to conventional chemicals (Daly & Fitt 1990; Duffield 2004) and seems to be working to hold back the development of resistance to the Cry toxins (Naranjo *et al.* 2008). However, with restricted water

availability due to the drought, pupae busting conflicts with minimum tillage strategies to maintain soil structure, reduce nitrogen applications, and capture more moisture in the soil. These factors also impact on the management of weeds, fungi and pathogens, so pupae busting could impact on the IPM of non-insect pests. As IPM develops by becoming more integrated within the total cropping system, overcoming conflicts such as these are important to the advancement of IPM within Australian cotton.

POTENTIAL FUTURE DIRECTIONS.

Currently in Australia only Monsanto has transgenic cotton in the market. Bollgard II[®] cotton expresses both Cry1Ac and Cry2Ab proteins. However, while the estimated frequency of alleles conferring resistance to Cry1Ac in Australia is very low at <0.0003, the estimated background frequency of alleles conferring resistance to Cry2Ab is substantially higher at 0.0033 (Mahon *et al.* 2007). The frequency for resistance to the Cry2Ab toxin is much higher than expected, and may put extra pressure on the pre-emptive resistance management strategies in place. In particular, it may be necessary to increase the amount of refuges available to increase the proportion of moths in the environment not exposed to Bt cotton, or it may be necessary to put a cap on the overall amount of Bollgard II[®] cotton that can be planted. This would strongly impact on the industry as it would mean that a higher proportion of cotton would again be susceptible to *Helicoverpa* spp. and require pesticide applications to manage this pest (Gregg & Wilson 2008). The challenge would be to maintain the IPM advances gained in light of the increased *Helicoverpa* threat. (the Cry2Ab frequency may also have nothing to do with Bt cotton)

The introduction of other types of transgenic insecticidal cotton (Table 1) could reduce the resistance pressure on Bollgard II[®] cotton. Whether any are marketed in Australia depends on performance against *H. armigera*, regulatory approval and a marketing mechanism. Any product which relies on a single mode of action such as Vipcot[®] or Widestrike[®], would come under some scrutiny for resistance management similar to Ingard[®] from 1996 to 2003, where a cap of 30% was required while Bollgard II[®] was developed. The list in Table 1 shows a heavy reliance on Cry1A proteins.

Vipcot[®] cotton, developed by Syngenta, uses an insecticidal protein produced during the vegetative growth phase of *Bacillus thuringiensis*, so-called vegetative insecticidal protein. This has a totally different mode of action to both Cry1Ac, and Cry2Ab, but it has similar efficacy against *Helicoverpa* spp (Liao *et al.* 2002; Llewellyn *et al.* 2007; Whitehouse *et al.* 2007). The effect of this transgenic variety on the cotton invertebrate community is similar to that of Bt cotton, with crop type explaining about 5% of the variance between the Vipcot[®] and conventional cotton communities. The number of predatory beetles and mirids were higher in Vipcot[®], although the increase in mirids was probably the result of more food (bolls) in the Vipcot[®] crop (Whitehouse *et al.* 2007).

Table 1. Transgenic insecticidal cotton varieties that may become available in Australia and the lethal proteins expressed by the genes inserted into these varieties.

Company	Trait-product	Gene #1	Gene #2
Monsanto	Bollgard II®	Cry 1Ac	Cry 2Ab
Bayer	Twinlink®	Cry 1Ab	Cry 2Ae
Syngenta	Vipcot®	Cry 1Ab	VIP
Dow	Widestrike®	Cry 1Ac	Cry 1F

Bayer is developing Twinlink® which contains genes for the Cry1Ab and Cry2Ae proteins, and the *bar* gene from the bacterium *Streptomyces hygroscopicus* (Jensen) (Actinobacteria: Streptomycetaceae) encoding the enzyme phosphinothricin acetyltransferase (PAT) which provides tolerance to herbicides containing glufosinate ammonium. This product is very similar to Bollgard II® as Cry1Ab competes for the same binding sites as Cry1Ac (Li *et al.* 2004); although it is unclear if Cry2Ae competes with the same binding sites as Cry2Ab. If it does use the same binding sites, then Twinlink® will not provide any relief to the resistance pressure on Bollgard II®.

Another transgenic that could come to Australia is Widestrike® (Dow Agrosciences). It too expresses Cry1Ac, but also Cry 1F. Cry 1F is particularly effective against armyworms (*Spodoptera* spp., Adamczyk & Gore 2004) but less effective against *H. armigera*, (Liao *et al.* 2002). In addition, although binding affinities are unknown in *H. armigera*, Cry1Ac competes for 60% of Cry1Fa binding sites in *H. zea* (Storer 2005). Thus it appears that this transgenic would only provide limited relief to the resistance pressure on Bollgard II.

However Widestrike®, perhaps in combination with another transgenic, may be an attractive option in new cotton growing regions opening up in the north (to make use of the water available in that region) such as the Burdekin (Grundy & Yeates 2007). Cotton, if it is viable in the Burdekin, will probably be exposed to more *Spodoptera*, which would make Widestrike® more attractive, but Widestrike® would only provide one gene to control *Helicoverpa*, causing a potential resistance problem. Ideally, Widestrike® needs to be combined with either Vipcot® or Twinlink® to be competitive and effective in the north. Thus as new transgenics become available, different regions of Australia may use different transgenics. The implications, in terms of the subtle changes to the natural enemy communities in cotton crops and in terms of resistance management, are at this stage unclear. As all the transgenic cottons potentially available contain a Cry1A gene, the best policy may be to treat all transgenics as one entity for resistance management strategies such as refuges.

The sustained drought over much of eastern Australia for the last few years is also applying pressure for agronomic change. As part of this, cotton no longer dominates, but is increasingly one in a mosaic of crops. This has ramifications for IPM and AWM, both of which are more developed in the cotton industry than in other crops. A wide range of crops can help IPM be more successful as they spread pest pressure and provide alternative sources for beneficials. However, they also complicate matters as pests can build up in one crop and then move into an adjacent

crop (Fitt *et al. in press*). Work is underway to engage with other cropping industries to co-ordinate IPM across the agroecosystem.

CONCLUSIONS.

Pressure from the drought has supported an increasing emphasis on conservation and sustainability within Australian cotton (2008 Australian Cotton conference). As part of this push, the Australian cotton industry is establishing itself as ecologically friendly and sustainable through the BMP (Best Management Practice) brand, where Australian cotton is labelled “BMP cotton” if its production conforms to environmentally and ethically responsible requirements such as best water management practices, healthy soils, and the best IPM practices (see website: <http://www.bmpcotton.com.au/>). This branding provides additional industry support to further develop IPM in Australian cotton. Transgenic cotton has a central role in this development as it provides conditions that enhance IPM by largely controlling *Helicoverpa* damage. The challenge will be to balance IPM and sustainable agronomy with the need to restrict the development of resistance by *Helicoverpa* to transgenic insecticidal cotton.

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IPM IN ECOLOGICAL ENGINEERING: USING PEST-RESISTANT CROPS WITH SUSTAINABLE BIOLOGICAL CONTROL

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ABSTRACT.

The use of pest-resistant crop varieties (conventionally bred and GM) has underpinned the development of IPM in many cropping systems. I will use examples of conventional and GM aphid resistant crops to illustrate how ecological research can go beyond biosafety assessments and help to optimise the use of pest-resistant crops to in current agricultural systems faced with combined challenges of reduced pesticide inputs, increased pest pressure and climate change.

INTRODUCTION.

Pest-resistant crop varieties have long formed the foundation of most IPM systems, theoretical and applied (Gowling & van Emden, 1993). Optimal deployment of pest-resistant crops with biocontrol agents requires a sound understanding of the mechanisms of resistance (direct and indirect), their effects on target pests and interactions (positive, negative or neutral) on key natural enemies in the agro-ecosystem (Birch & Wheatley 2005). In a recent survey of 62 IPM initiatives in 26 developing and industrialised countries, Petty (2005) found that over 60% fell into the category of reduced pesticide inputs coupled with increased yields, whilst only one case illustrated a complete failure of IPM (pesticide use increase but yield decreases). In developing countries particularly, IPM can bring pesticide reductions of up to 65% without reducing yields (Petty 2005). However, looking in more detail at the ecological interactions in agro-ecosystems, Groot & Dicke (2002) found that 33% of laboratory and field based studies on pest-resistant GM crops showed negative impacts on biocontrol agents (parasitic and predatory natural enemies), broadly similar to non-target effects of pest-resistant conventionally bred crops (14%). Unfortunately, plant breeding for pest resistance and biocontrol research are still often carried out independently, hindering development of optimised IPM based on host plant resistance (Courtesero, Stapel & Lewis 2000). A more holistic approach offers the potential of using networks of interactions in agro-ecosystems to optimise IPM using both bottom up and top down forces together with managed habitats to suppress pests and diseases below economic thresholds in a more sustainable manner (Gurr, Wratten & Altieri 2004; McCann, 2007).

METHODS.

We have developed several methodologies to focus our research on the key interactions beneficial to IPM in agro-ecosystems. These include the 'funnel' selection methodology (Birch *et al.* 2004; Hilbeck *et al.* 2008), holistic ecological/ecotoxicological hybrid methods (Birch *et al.* 2007) and food web analysis (e.g. Paine 1992). See cited papers and book chapters for a full description. This involves the

characterisation of food webs in regional agro-ecosystems, using pre-selected criteria listed in selection matrices (e.g. importance in biocontrol to suppress key pests in the region) in order to prioritise the key ecological interactions for detailed study, rather than attempting to study all the interactions in a more superficial manner.

RESULTS.

Case studies in Kenya, Brazil and Vietnam (Birch *et al.* 2004; 2006; 2008) were used to develop and validate a 'funnel' methodology for prioritising key functional groups and species in the assessment in risk:benefit of *Bt* crops. An added benefit of this approach, apart from facilitating more cost-effective studies of complex ecosystems, is to focus research on understanding the key interactions in food webs which are most important for optimising biocontrol when used in combination with pest-resistant crop varieties. Further studies (laboratory, glasshouse and field) on *Bt* maize showed a lack of predictability and translation across the three most commonly used testing scales (tiers) and re-enforced our belief that a more holistic approach, incorporating feedback loops within and between experimental scales, gives more accurate information about the positive, negative or neutral effects of pest-resistant crops on agro-ecosystems.

DISCUSSION.

Our research on GM crops has been part of large, multi-national projects in which methodologies have been developed and validated at different scales and in several different agro-ecosystems and environments. The robustness and flexibility of these methods has been validated and is currently being used to optimise the use of aphid-resistant raspberry varieties in two differing environments (open field and protected polytunnels) where initial studies are characterising very different food web structures in terms of species diversity, abundance and stability over changing temporal and spatial conditions. This approach will be used to identify the most important interactions in each food web and should allow us to introduce crop management practices which provide more sustainable and effective IPM, at a time when many pesticides are being withdrawn by the EU and when the threat of pest attack is at the same time increasing due to climate change and new methods of production.

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POTENTIAL IMPACTS OF GENETICALLY-MODIFIED TREES ON BIOLOGICAL CONTROL AGENTS IN PLANTATION FORESTS

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ABSTRACT.

Genetic modification of forest trees with insecticidal genes could provide long-term protection from attack by serious defoliating pests. However, their non-target impacts need to be understood to ensure that natural biological control of other herbivorous arthropods is not disrupted. Cry1Ac toxin from *Bacillus thuringiensis* and avidin from chicken egg white are two different insecticidal proteins that are candidates for incorporation into transgenic trees. Laboratory-based experiments with foliage-feeding pests of New Zealand plantation forests have shown that, when expressed in transgenic plants, both traits may negatively affect insects such as the geometrid *Pseudocoremia suavis* and the tortricids *Ctenopsuestis obliquana* and *Epiphyas postvittana*. Tri-trophic studies with Cry1Ac- and avidin-expressing plants and two natural enemies of New Zealand forest invertebrates, *Meteorus pulchricornis* (a braconid parasitoid) and *Ctenognathus novaezelandiae* (a carabid predator) respectively, showed that the transgenic traits had minor negative impacts on these insects, and suggested that their main impact would be via reductions in the populations of hosts and prey. The planting of non-transgenic refuges for natural enemies could be a useful strategy for maintaining biological control in transgenic plantation forests.

INTRODUCTION.

Defoliating insects can seriously threaten timber and pulp production from plantation forests in many countries. For this reason major pest species such as *Lymantria dispar* (L.), *Teia anartoides* Walker and *Orgyia thyellina* Butler (Lepidoptera: Lymantriidae) are regarded as significant biosecurity threats to New Zealand pine plantations and expensive monitoring and eradication programmes have been conducted to avert these exotic invaders (Brockerhoff *et al.* 2006; Hosking *et al.* 2003; Suckling *et al.* 2005). Genetic modification of forest trees with insecticidal genes, such as those from *Bacillus thuringiensis* Berliner (*Bt*), could offer built-in, long-term protection against pest attack (Grace *et al.* 2005; Lachance *et al.* 2007). However, the need to control herbivorous invaders through GM-trees expressing insecticidal traits must be weighed against the potential impacts of these trees on non-target organisms, such as herbivores normally kept at sub-pest levels by their natural enemies.

Biological control can be important in forests, where economic pest thresholds are often high compared with other agricultural systems, and suites of natural enemies can effectively suppress populations of plant-feeding insects. This is

illustrated by the case of the common forest looper, *Pseudocoremia suavis* (Butler) (Lepidoptera: Geometridae), in New Zealand pine plantations. This endemic geometrid underwent population explosions following drought conditions in the 1950s and 1970s, causing extensive defoliation of *Pinus radiata* D. Don (Pinales: Pinaceae, Monterey pine) and *Pseudotsuga menziesii* (Mirb.) Franco (Pinales: Pinaceae, Douglas fir) plantations (Kay 1982; 1983; White 1974). Insecticidal sprays were employed during these outbreaks, but in some stands of trees epizootics of a cytoplasmic polyhedrosis virus caused the insect populations to collapse before the sprays could be applied. An entomopathogenic fungus and a nucleopolyhedrosis virus, which may also have contributed to the pest's decline, were found (Moore & Alma 1974; White 1974). Currently, *P. suavis* and at least four other species of native geometrids are commonly found in commercial pine plantations but they are apparently effectively controlled by parasitoids and diseases (Berndt *et al.* 2006; Moore & Alma 1974; White 1974). Recent faunal surveys of New Zealand pine plantations have revealed high numbers of predatory arthropods, such as spiders, and these may also play important roles in suppression of herbivorous pests.

This paper describes the potential impacts of transgenic trees expressing two different insecticidal toxins on herbivorous insects and their natural enemies in plantation forests in New Zealand (Table 1).

Table 1. Laboratory-based experiments to investigate impacts of insect-resistant transgenic plants on New Zealand plantation forest-dwelling insect species.

Gene	Test plant	Herbivore	Natural enemy
Cry1Ac (<i>Bt</i>)	<i>Pinus radiata</i>	<i>Pseudocoremia suavis</i>	
Cry1Ac (<i>Bt</i>)	<i>Pinus radiata</i>	<i>Ctenopseustis obliquana</i>	
Cry1Ac (<i>Bt</i>)	<i>Pinus radiata</i>	<i>Pseudocoremia suavis</i>	<i>Meteorus pulchricornis</i>
Avidin	<i>Eucalyptus grandis</i>	<i>Epiphyas postvittana</i>	
Avidin	<i>Nicotiana tabacum</i> (model plant)	<i>Spodoptera litura</i> (model insect)	<i>Ctenognathus novaezelandiae</i>

MATERIALS & METHODS.

The impacts of two insecticidal traits for transgenic trees on New Zealand forest insects have been investigated: the *Bt* toxin Cry1Ac and the biotin-binding protein avidin. Both proteins are well-known for their insecticidal properties; Cry1Ac is lethal when consumed by larvae of many lepidopteran species (Glare & O'Callaghan 2000) and avidin stunts and kills the larvae of a wide range of herbivorous insect species (e.g. Burgess *et al.* 2001; 2002; Kramer *et al.* 2000; Markwick *et al.* 2001).

Experiments with Cry1Ac.

Plants. *Bt*-expressing transgenic *P. radiata* trees were produced using biolistic transformation to insert the *cry1Ac* gene, the *nptII* selectable marker gene, and the *uidA* reporter gene into embryonic tissue as described by Grace *et al.* (2005). Plants were maintained in a glasshouse under natural light:dark conditions at approximately 18-20°C. Foliage was harvested from these trees and from isogenic, non-transformed control trees for use in experiments with insects.

Insects. Larvae of the native looper *P. suavis* were obtained from a laboratory colony founded from larvae and adults collected from Woodhill Forest, a commercial pine plantation 30 km north of Auckland, New Zealand. The colony was initially line-bred to eliminate any entomopathogens and was thereafter maintained at HortResearch, Auckland, on surface-sterilized foliage from non-transgenic *P. radiata*. Larvae of the native leafroller *Ctenopseustis obliquana* (Walker) (Lepidoptera: Tortricidae) were obtained from a laboratory colony at HortResearch, Auckland, where they were reared on general purpose diet (GPD) (Singh & Moore 1985).

For tri-trophic experiments, a laboratory colony of the self-introduced parasitoid *Meteorus pulchricornis* (Wesmael) (Hymenoptera: Braconidae) was maintained by rearing them on *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), which were fed on GPD until parasitoid emergence. The *M. pulchricornis* colony founders had emerged from *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae field-collected from Hawkes Bay, New Zealand. Parasitoids were kept in 3L clear plastic jars at ambient summer/autumn temperatures and light regimes, with access to honey and 10% w/v sugar solution. Female *M. pulchricornis* adults, between two and four weeks old and previously exposed only once to *S. litura* hosts, were used to parasitise pine-fed *P. suavis* in tri-trophic experiments.

Impacts of *Bt* pine on herbivores. The effects of *Bt* pine on *P. suavis* growth and survival were investigated by supplying neonate larvae with foliage from transgenic Cry1Ac-expressing pines (eight plants from a single transformation event) or with non-transgenic control pine foliage. Each larva was kept in a separate 4mL plastic container, at 20°C, in darkness for one week and under a 16:8 h light:dark regime thereafter. All insects were checked three times per week and supplied with more foliage of the same type *ad libitum*. Larval weights were recorded weekly from 14 days onwards and pupae were weighed. Days to pupation or death were recorded.

In a similar experiment, second instar *C. obliquana* larvae were fed until death or pupation on foliage from pines expressing Cry1Ac (plants from four separate transformation events in two different genotypic backgrounds) or non-transgenic control pine trees (two lines, isogenic with the two genotypic backgrounds used in the transformations). Larvae were fed *ad libitum* with foliage from each of the six groups of plants (four expressing Cry1Ac and two controls). Larvae were checked three times per week for survival, pupation and adult emergence and were weighed every seven days and after pupation.

Tri-trophic impacts of *Bt* pine on a parasitoid. Second instar *P. suavis* larvae, raised on either control non-transgenic pine or *Bt* pine (single transformation event) foliage, were each exposed to *M. pulchricornis* adults until oviposition was observed.

Each larva was then reared individually on the same type of pine foliage and checked daily for pupation, parasitoid emergence and/or death. Because *P. suavis* grew more slowly on *Bt* pine than on control foliage, early rearing temperatures and times were adjusted so that host larvae of similar sizes could be selected for parasitism, in order to minimise the possibility that *Bt*'s effects on host size would be a primary influence on the outcomes observed.

Pseudocoremia suavis that died as larvae were dissected and examined for head capsules of *M. pulchricornis* in order to eliminate any individuals that had escaped parasitism from the analysis. *Meteorus pulchricornis* larvae that emerged from hosts spun cocoons and pupated within them. These cocoons were weighed when they were four days old. Adult parasitoids that emerged from their cocoons were kept individually in 50mL containers, fed with honey and sugar solution (10% w:v), and checked daily for survival. After 41 days, a sub-sample of the surviving adults were each confined with some second instar *S. litura* larvae to check for fertility. The remaining adult *M. pulchricornis* were killed by freezing, their ovaries dissected and their eggs counted (potential fecundity).

Experiments with avidin.

Impacts of avidin eucalyptus on a herbivore. *Eucalyptus grandis* W. Hill ex Maid. (Myrtales: Myrtaceae) plant explants were transformed with a construct comprising an avidin gene, a CaMV 35S promoter, and a vacuolar-targeting leader sequence from potato protease inhibitor 1 using standard *Agrobacterium tumefaciens* methods. Leaves expressed 64-275 ppm avidin. Rooted plantlets were transferred to potting mix and plants were grown to a height of 0.75 to 1.5 m in a glasshouse at 15-22°C, 16:8 h light:dark. Neonate larvae of *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) were obtained from a laboratory colony at HortResearch, Auckland, that had been maintained as described above for *C. obliquana*. Larvae were placed individually in 4mL containers with excised pieces of young *E. grandis* leaves from non-transgenic control or transgenic avidin-expressing plants, kept at 20°C, 16:8 h light:dark, and checked and weighed regularly.

Tri-trophic impacts of avidin tobacco on a predator. *Nicotiana tabacum* L. (Solanales: Solanaceae) plants were transformed via standard *A. tumefaciens* methods with the avidin construct described above and grown in a glasshouse at 24°C, 16:8 h light:dark. Adults of the endemic carabid beetle *Ctenognathus novaezealandiae* (Fairmaire) (Coleoptera: Carabidae) were collected from Woodhill forest and maintained in the laboratory (Philip & Burgess 2008a). Adults, and larvae raised from eggs laid by field-collected adult beetles, were used in a series of feeding trials, where they were supplied with prey comprising small larvae of *S. litura* that had been raised on transgenic avidin-expressing (55-67 ppm) or non-transgenic control tobacco leaves, and/or field-collected forest floor invertebrates (mainly amphipods, isopods and bristletails). In these extended experiments (91 days for beetle larvae, 280 days for adult beetles fed exclusively on *S. litura*, and 360 days for adults fed with mixtures of *S. litura* and field-collected prey), beetle weights, survival, fecundity, fertility and consumption of food were recorded (Burgess *et al.* in press; 2008; Philip & Burgess 2008a; 2008b).

RESULTS.

Experiments with Cry1Ac.

Impacts of Bt pine on *Pseudocoremia suavis*. *Pseudocoremia suavis* larvae fed with Cry1Ac-expressing *P. radiata* had significantly poorer survival to pupation than those fed with non-transgenic pine (28% vs 68% survival, respectively; $\chi^2 = 7.9$, $df = 1$, $P = 0.005$). Bt-pine-fed *P. suavis* took significantly longer to pupate (42.3 ± 1.5 days) than the controls (31.6 ± 0.9 days) ($F_{1,20} = 36.08$, $P < 0.0001$), but had similar pupal weights.

Impacts of Bt pine on *Ctenopseustis obliquana*. One of the lines of Cry1Ac-expressing transgenic pine (Bt gus2 99-76) significantly suppressed larval growth and killed all *C. obliquana* before pupation (Fig. 1) ($\chi^2 = 8.47$, $df = 1$, $P = 0.004$, Minitab 15 log-rank test). A second Bt line (Bt6 94-18) slowed larval growth (ANOVA, Tukey comparison mean weights, $P = 0.0045$ on days 7 and 14, $P = 0.004$ on day 21), delayed pupation (ANOVA, mean time to pupation, $P < 0.0005$) reduced pupal weight (ANOVA, Tukey comparison, $P < 0.0065$) and may have caused increased larval and pupal death. The other two Bt lines had no discernable effect on larvae.

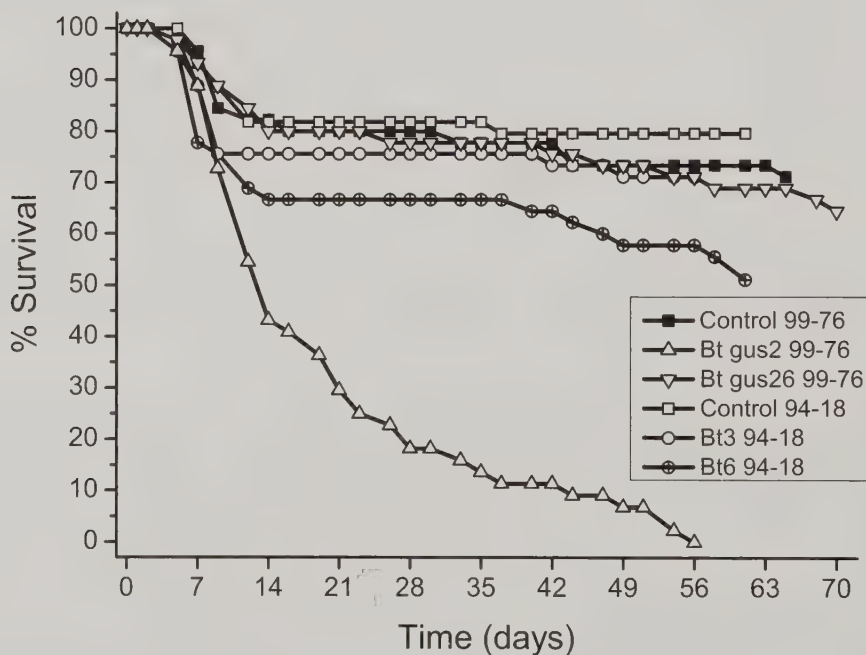


Fig. 1. Survival to adulthood of *Ctenopseustis obliquana* fed as larvae on needles from two control and four Bt-modified lines of *Pinus radiata*.

Tri-trophic impacts of Bt pine on *Meteorus pulchricornis*. When both pre- and post-emergence survival were considered together, parasitoids with Bt-pine-fed hosts had significantly poorer survival than parasitoids with hosts that had fed on control pine, (49% survival in Bt-fed hosts vs 64% in control-pine-fed hosts; $\chi^2 = 5.2$, $df = 1$, $P = 0.023$). Observed pre-emergence host mortality in parasitized Bt pine-fed *P.*

suavis was consistent with the level of host mortality expected if parasitism, which itself caused significant pre-emergence host death, and the Bt toxin were acting independently as larval mortality factors. Parasitized hosts fed on Bt pine had significantly reduced growth rates (Fig. 2) and parasitoids took significantly longer to emerge from hosts in the Bt group (19.6 days vs 17.9 days, respectively; $F(1,108) = 11.99$, $P < 0.001$). Ingestion of Bt-pine by *P. suavis* hosts did not affect *M. pulchricornis* pupal duration, weight of pupa in its cocoon, or the number of eggs in F_1 parasitoid adults' ovaries. There were no differences in the longevity of F_1 *M. pulchricornis* adults over the 41 days of the experiment, or in the ability of survivors to successfully parasitise *S. litura*, suggesting that adult longevity and fertility had not been affected by larval exposure to Bt-pine-fed hosts.

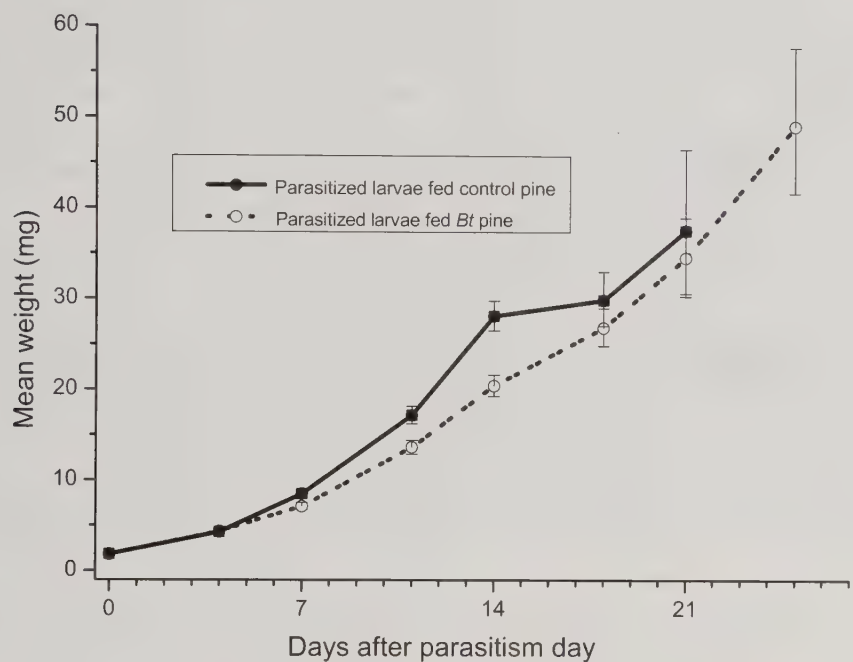


Fig. 2. Mean weights of *Pseudocoremia suavis* larvae parasitised by *Meteorus pulchricornis* and fed with control or Bt-modified *Pinus radiata*.

Experiments with avidin.

Impacts of avidin eucalyptus on Epiphyas postvittana. Avidin-expressing *E. grandis* trees were highly resistant to *E. postvittana*. Larvae fed with avidin-expressing leaves had significantly reduced mean weights compared with those on non-transgenic control leaves (0.56 ± 0.16 mg vs 4.94 ± 1.18 mg, respectively; $P < 0.001$, Genstat REML analysis) and their median longevity was significantly reduced (21 days vs 30 days; $\chi^2 = 13$, $df = 1$, $P = 0.0003$).

Tri-trophic impacts of avidin tobacco on Ctenognathus novaezealandiae. Only minor differences in the performance of *C. novaezealandiae* could be attributed to the tri-trophic influence of avidin expression. *C. novaezealandiae* larvae that been fed from hatching with an exclusive diet of *S. litura* larvae raised on transgenic avidin-expressing (67 ppm) tobacco grew faster and were significantly larger than beetle

larvae fed with control-tobacco-fed prey (mean larval weights of 10.5 mg vs 9.5 mg, respectively; ANOVA, $P < 0.001$). There were no effects of the prey's diet on larval beetle survival or rates of food consumption. Newly-emerged adult *C. novaezealandiae* fed exclusively on *S. litura* larvae fed with either avidin-expressing (55 ppm) transgenic tobacco or with non-transgenic control tobacco did not differ significantly from each other in terms of weight changes, survival or fecundity. Beetles given only avidin-tobacco-fed prey consumed significantly less than those given only control prey between Days 77 and 280 (150 mg vs 156 mg per beetle per week; $F_{2,27} = 6.11$, $P = 0.0065$) (Burgess *et al.* 2008). Adult beetles given mixtures of forest floor invertebrates and tobacco-fed *S. litura* performed better than those given only tobacco-fed prey in terms of fecundity, egg fertility, body weight and male beetle survival. Fewer of the avidin tobacco-fed prey were consumed than the control tobacco-fed, whatever mixture or proportion offered (71.3-71.9 g vs 74.5-75.2 g per beetle per feed, respectively; $F_{1,26} = 202.55$, $P < 0.001$). This was probably a result of the reduced quality of biotin-deprived prey. Beetles consuming 100% avidin tobacco-fed prey had lower fecundity than those given 100% control tobacco-fed prey (0.019 vs 0.094 eggs per female beetle per week, respectively; $t(26) = 2.87$, $P = 0.008$), although predation on eggs could have contributed to this result. Despite the nutritionally limiting nature of an exclusive diet of tobacco-fed prey, there was no effect of avidin on fecundity in beetles consuming 67% or 33% avidin prey, nor any effect on female or male mass, survival or egg fertility, even in the 100% avidin prey treatment. Fecundity in beetles fed 33% field food with 67% tobacco-fed prey was lower than in those fed 67% or 100% field food (0.49 vs 1.42 or 1.26 eggs per female per week; $t(26) = 6.99$, $P < 0.001$ or $t(26) = 2.87$, $P < 0.001$, respectively). However, there was no added impact of avidin on fecundity, mass or survival, or egg fertility of the 33% field food diet, suggesting that under field conditions, where a mixture of prey is available, negative impacts of avidin-fed prey are unlikely (Burgess *et al.* in press).

DISCUSSION.

Results obtained so far suggest that Cry 1Ac-expressing pine trees will be compatible with the biological control currently exerted on the herbivorous lepidopterans, *P. suavis* and *C. obliquana*, by the generalist parasitoid *M. pulchricornis* in New Zealand. The transgenic trees are likely to cause reductions in the numbers of *P. suavis* larvae, and perhaps greater reductions in the numbers of the leafroller *C. obliquana*, but it is likely that non-transgenic refuges planted for *Bt* resistance management and neighbouring stands of other tree species will ensure that some of these polyphagous caterpillars survive to act as hosts for the parasitoid. As *M. pulchricornis* is a generalist, it could conceivably also use alternative host species to maintain its populations. Pupation was delayed by about 10 days in *P. suavis* that survived their exposure to Cry1Ac as larvae. Such a delay might affect parasitoid and host survival in the field, perhaps via extended exposure to other natural enemies such as predators, viruses or microsporidia.

The potential compatibility of *Bt* transgenic trees with biological control indicated by these results accords with observations from other transgenic *Bt*-expressing crops (Romeis *et al.*, 2008). For example, Naranjo (2005) found that even though predators became less abundant over five years with *Bt*-cotton crops, due to the reduction in prey numbers (i.e. pest control), predator:prey ratios for pest species such as *Lygus hesperus* Knight (Hemiptera: Miridae) and *Bemisia tabaci* (Gennadius) (Hemiptera:

Aleyrodidae), which are not susceptible to *Bt*, did not differ between unsprayed *Bt*- and non-*Bt*-cotton.

Avidin has a much broader spectrum of insecticidal activity than *Bt*, effective against Lepidoptera, Coleoptera, Orthoptera, Diptera and mites (Burgess *et al.* 2001; 2002; Kramer *et al.* 2000; Levinson & Bergmann 1959; Levinson *et al.* 1992; Malone *et al.* 2002; Markwick *et al.* 2001; 2003; Morgan *et al.* 1993). Thus we would expect population reductions in a larger number of foliage-feeding arthropod species on avidin-expressing trees than on Cry1Ac-expressing trees. However, because expression of avidin in transgenic plants is directed to the vacuoles (constitutive expression of avidin would create a lethal biotin deficiency in the plant itself), only arthropods able to gain access to and feed on vacuolar contents may succumb to its effects. Therefore the impacts of avidin-expressing transgenic trees will probably be limited to members of particular feeding guilds, such as leaf chewers and plant-cell-content suckers. Even within these groups there may be interspecific differences in susceptibility to avidin (Markwick *et al.* 2001). Furthermore, since avidin-induced biotin deficiency is reversible (Morgan *et al.* 1993), some generalists may also be able to escape its negative effects by obtaining biotin from other food sources.

Although there have been no direct studies of the impacts of avidin consumption by hosts on their parasitoids, it is unlikely that parasitoids will be directly exposed to avidin at insecticidal levels. Measurements of avidin activity in the bodies of herbivores feeding on transgenic avidin-expressing plants indicate that there is a reduction in the concentration of avidin compared with the level found in the plants which is consistent with active avidin being confined to plant material within the insect's gut rather than being absorbed into the haemocoel (Christeller *et al.* 2005). Thus we may speculate that the major impact of avidin-expressing trees on parasitoids will be via a reduction in the numbers of available hosts. Further research is required to determine whether this impact on host numbers would be large enough to threaten the current effectiveness of these natural enemies.

Our results with the carabid beetle suggest that generalist predators will not be unduly affected by their prey consuming avidin, and even reductions in the numbers of leaf-chewing arthropods may not be a threat provided sufficient alternative prey is available. Once again, a good understanding of the field biology of the protagonists will be important in predicting impacts of transgenic insect-resistant trees on natural biological control in forests.

The use of transgenic insect-resistant trees could provide built-in, long-term protection against pest threats to many plantation forests. The potential for impacts on non-target organisms such as natural enemies will have to be considered on a case-by-case basis and will vary with the types of traits being deployed and the complexes of species in the forests. Results so far suggest that planting refuges of non-transgenic trees may be a sensible strategy to preserve not only the efficacy of traits like *Bt* Cry toxins, but also any organisms responsible for natural biological control of herbivorous insects.

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COMPATIBILITY OF BIOLOGICAL CONTROL WITH LEPIDOPTERAN-PROTECTED *Bt* CORN

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ABSTRACT.

Insect-protected transgenic crops like Bt corn that express insecticidal proteins derived from *Bacillus thuringiensis* (Bt) have the potential to complement the aims and tools of integrated pest management (IPM). The first Bt corn hybrids engineered to control lepidopteran corn pests were commercialized in 1996 in the USA. These Bt corn hybrids have since been adopted globally on tens of millions of hectares. Because of testing of these products prior to and post-commercialization, a substantial body of literature now exists describing laboratory and field studies focused upon assessing the potential and realized impacts of lepidopteran-protected Bt corn products on non-target organisms. Collectively, these studies indicate that lepidopteran-protected Bt corn hybrids have no consistent adverse effects on non-target species in general and on biological control agents in particular. Furthermore, the ability of these technologies to replace the use of broad-spectrum conventional insecticides which have documented effects on natural enemies suggests that the global adoption of Bt corn products should tend to enhance biological control in corn agro-ecosystems.

INTRODUCTION.

Insect-protected transgenic crops like Bt corn that express insecticidal proteins derived from *Bacillus thuringiensis* (Bt) have the potential to complement the aims and tools of integrated pest management (IPM). The insecticidal specificity and effectiveness of Bt proteins are well documented from work on in vitro systems and for the same proteins used as foliar products (English & Slatin 1992). Each Bt protein only affects a relatively small set of related insect species and unrelated non-target species are unaffected. The first Bt corn hybrids were commercialized in 1996 in the USA (Mendelsohn *et al.* 2003; James 2007). These products contained the Bt protein Cry1Ab and were targeted at lepidopteran pests of corn, particularly stem borers such as the European corn borer *Ostrinia nubilalis* and ear-feeding insects such as the corn earworm *Helicoverpa zea* that are difficult to control using conventional insecticides. Subsequently, a suite of comparable Bt corn products containing other lepidopteran-active Bt proteins such as Cry1F and Cry2Ab2 have been introduced. These Bt corn hybrids have since been adopted on tens of millions of hectares globally, with the highest adoption occurring in the USA, Canada, Argentina, Colombia, Spain, South Africa and the Philippines (James 2007).

Prior to commercialization, each of these products underwent extensive environmental testing as part of their safety assessment by regulatory authorities

such as EPA and USDA (Romeis *et al.* 2008). This testing involved laboratory-based studies with a range of representative non-target organisms. After commercialization, these same products were examined at progressively larger scales in the field by private and public sector scientists (e.g., see Naranjo *et al.* 2005). Consequently, a substantial body of literature now exists describing laboratory and field studies focused upon assessing the potential and realized impacts of Bt corn products on non-target organisms. This paper provides a brief review of this literature with a particular focus on what it tells us about the ability of Bt corn products to complement biological control in corn agro-ecosystems.

EFFECTS OF LEPIDOPTERAN-PROTECTED BT CORN ON NON-TARGET ORGANISMS.

By facilitating reduced use of broad-spectrum insecticides against pests like European corn borer, Bt corn products provide a number of environmental benefits in addition to economic benefits for farmers. Bt corn products, with their high insecticidal specificity and reduced insecticide use, allow greater survival of a range of beneficial non-target species, including generalist predators that are important for biological control of secondary pests and pollinators. These impacts will be realized both within Bt corn fields and in neighboring fields through reduced insecticide drift. These environmental benefits have been comprehensively demonstrated through a large number of laboratory and field studies that have looked at the ecological impacts of Bt corn and alternative insect-control practices on non-target invertebrate populations and communities (Candolfi *et al.* 2004; Daly & Buntin 2005; de la Poza *et al.* 2005; Naranjo *et al.* 2005; Pilcher *et al.* 2005; Romeis *et al.* 2006; Marvier *et al.* 2007). In many cases, these studies were part of applications for regulatory approval and were conducted prior to the commercialization of products (Mendelsohn *et al.* 2003). However, for some of the large-scale, multi-year field studies, the work was conducted in commercial fields under typical farmer practices (e.g., Naranjo *et al.* 2005; de la Poza *et al.* 2005). These studies have involved field corn and sweet corn, and a variety of Bt proteins (including Cry1Ab, Cry1F and VIP3A) and bio-climatic zones (including US, Spanish and French cropping systems). Collectively, they demonstrate that Bt corn products do not have unexpected toxic effects on non-target species; only the targeted pest species are directly impacted by Bt corn, as would be predicted from knowledge of the mode of action and specificity of Bt proteins (English & Slatin 1992; Mendelsohn *et al.* 2003; Naranjo *et al.* 2005; Romeis *et al.* 2006; Marvier *et al.* 2007).

The activity spectrum of the Cry1 proteins in currently available lepidopteran-protected Bt corn products is specific for Lepidoptera. This has been confirmed through extensive laboratory testing involving representative species from a variety of insect orders, including Lepidoptera, Diptera, Hymenoptera, Coleoptera, Hemiptera and Collembola (e.g., MacIntosh *et al.* 1990; Mendelsohn *et al.* 2003; Romeis *et al.* 2008). These tests typically involved exposing larvae in no-choice situations to high concentrations of the relevant Bt proteins in artificial diets. Initial reports suggested that the neuropteran *Chrysoperla carnea* might be susceptible to the Cry1Ab protein (Hilbeck *et al.* 1998a, b). However, subsequent tests using a variety of methods and

media determined that this was not the case for either larvae (Romeis *et al.* 2004) or adults of this species (Li *et al.* 2008).

The specificity of the Bt proteins present in Bt corn, together with the fact that they are contained within the corn plant tissues thereby minimizing the exposure of non-phytophagous insects to these proteins, means that Bt corn is not expected to have significant adverse impacts on non-target species in the field. As predicted, across the large number of field studies that have been conducted, few or no differences have been seen with respect to community structure or individual species abundance where fields of Bt corn have been compared to conventional corn that has not been treated with foliar insecticide sprays. Where they have been calculated (e.g., Lozzia 1999), indices of species diversity and community structure have not differed significantly for Bt cornfields compared to conventional cornfields. The only species that have been observed to be significantly and consistently less abundant in Bt cornfields than in conventional cornfields are the target pest Lepidoptera. In studies where some of the conventional corn fields have been sprayed for the target pest Lepidoptera of Bt corn, many non-target species have been observed to be adversely impacted, leading to significantly lower non-target populations, at least transiently, in sprayed conventional fields as compared to Bt corn fields (Candolfi *et al.* 2004; Naranjo *et al.* 2005; Marvier *et al.* 2007; Wolfenbarger *et al.* 2008). This is particularly obvious for foliage-dwelling species because of the method of application of these insecticides, but ground-dwelling species like carabids and cursorial spiders are also often affected, directly or indirectly, by the insecticidal sprays and are apparently not affected by Bt corn. Interestingly, when the impact of foliar sprays containing comparable Bt proteins were compared with the impacts of Bt corn, even the foliar sprays had greater adverse effects on non-target species, possibly because of other materials present within these sprays (Candolfi *et al.* 2004).

Many studies have concentrated on a particular species or guild, and natural enemies have received considerable attention because of their role in the biological control of various pests of corn. Based on what is known about the spectrum of activity of Cry1Ab and Cry1F, no direct toxic effects would be expected for any natural enemy species. However, indirect effects might occur if the prey or host species of the natural enemies were a target of Bt corn. Most of these studies have primarily looked at generalist predators, particularly *Coleomegilla maculata* (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Neuroptera: Chrysopidae), *Orius insidiosus* (Heteroptera: Anthracoridae), and guilds of carabids because of their abundance in cornfields and their perceived importance. No consistent effects of Bt corn have been seen for any of these species in these studies or in the broader, community-level studies (Lozzia 1999; Wold *et al.* 2001; Pilcher *et al.* 2005; Wolfenbarger *et al.* 2008). The absence of even indirect trophic effects of Bt corn in these studies is not surprising because these predatory species feed on many different prey species, the vast majority of which are not directly impacted by Bt corn e.g., sucking insects like aphids.

A smaller number of studies have looked at impacts on parasitoids or the level of parasitism in Bt cornfields. Because of their specificity, species that parasitize the target pests of Bt corn would be expected to be rarer in Bt corn. As expected, the few specialist parasitoids that parasitize *Ostrinia nubilalis* and certain other stalk boring Lepidoptera have been found to be rarer in Bt corn than in conventional corn e.g., *Macrocentrus cingulum* (Hymenoptera: Braconidae) (Candolfi *et al.* 2004; Pilcher *et*

al. 2005), though even this result is not consistent across studies or even within studies (Orr & Landis 1997, Pilcher *et al.* 2005).

As mentioned earlier, the insecticidal sprays used in conventional corn had clear adverse impacts, at least transiently, on almost all common natural enemies, both predators and parasitoids, and particularly those species foraging above-ground (Candolfi *et al.* 2004, Wolfenbarger *et al.* 2008). Therefore, the ability of Bt corn technologies to replace the use of broad-spectrum conventional insecticides which have documented effects on natural enemies suggests that the global adoption of Bt corn products should tend to enhance biological control in corn agro-ecosystems.

CONCLUSIONS.

The substantial body of literature on the non-target impacts of lepidopteran-protected Bt corn indicates that these products have no consistent adverse effects on non-target species in general and on biological control agents in particular. Furthermore, the ability of these technologies to replace the use of broad-spectrum conventional insecticides which have documented effects on natural enemies suggests that the global adoption of Bt corn products should tend to enhance biological control in corn agro-ecosystems.

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SESSION 6

IMPACT OF LANDSCAPE COMPOSITION AND STRUCTURE ON NATURAL ENEMIES

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Predators and parasitoids are critical control elements for pests in many crops and are often responsible for preventing outbreaks of pest populations. Frequently, a complex of natural enemies are responsible for causing pest mortality, but often not all the important species are identified, and their activity and habitat preferences unknown. Moreover, each natural enemy may have varying critical resource needs and therefore radically different plant preferences and responses to habitat composition. This may explain why augmentation or conservation attempts, especially through increases to plant diversity, have often been ineffective. Thus, there is a need for studying specific aspects of key natural enemies' ecology at a broader spatial scale than the crop. Lately, there has been significant progress in the characterization of natural enemy complexes impacting specific pests as well as determining the resource needs of significant natural enemy taxa and their varying responses to different crops, changes in crop diversity or habitat fragmentation. The objective of this session is to bring researchers together who are involved in current basic and applied research on the role of the habitat in mediating arthropod natural enemy abundance. Our broader goal is to improve our understanding of natural enemy response to habitat configuration and our ability to predict how modifications of the agricultural landscape might impact biological control of pests.

SPECIES SPECIFIC RESPONSE OF PREDATORS AND APHIDS TO HABITAT LOSS AND FRAGMENTATION IN EXPERIMENTAL ALFALFA LANDSCAPES

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ABSTRACT.

Habitat loss and fragmentation may affect the abundance of natural enemies, and their ability to control pests. It has been usually assumed that these processes have negative effects on the biota, but in studies where they are isolated, often habitat loss has negative effects on the abundance and diversity of organisms, while fragmentation has no effect, or even may have positive effects on population abundance and species richness. In this work we synthesized how habitat loss and fragmentation of experimental alfalfa crops affect the abundance and species richness of several arthropod predators and their prey, the aphids. During two consecutive years we generated landscapes with different combinations of habitat loss (0, 55 or 84%), fragmentation (1, 4 or 16 fragments) and isolation (0, 2 or 6 m between fragments). Our results suggest that high levels of habitat loss and fragmentation may affect the density and species richness of some predatory insects, although the responses vary over time and are species specific, with some species being more sensitive to landscape configuration than others. Most of the species that were sensitive to landscape structure were generally positively affected by habitat loss and/or fragmentation sometime during the season, with the exception of spiders, which were more abundant in unfragmented control landscapes. These results should be considered when designing agricultural landscapes in order to enhance biological control.

INTRODUCTION.

Habitat loss and fragmentation have been considered of great importance in conservation biology of natural ecosystems, but these processes are also relevant in agroecosystems because of their potential effects on predator-prey dynamics, and therefore, in the impact that natural enemies can have in suppressing pests (Kruess & Tscharntke 1994; Thies & Tscharntke 1999; Tscharntke & Kruess 1999; Hunter 2002; With *et al.* 2002). Habitat fragmentation may adversely affect the ability of natural enemies to control pest outbreaks in agricultural landscapes by interfering with their searching behavior and their aggregative numerical response to prey (Kareiva 1987; With *et al.* 2002). Additionally, it has been shown that species belonging to higher trophic levels, such as parasitoids and predators, are more affected by habitat loss and fragmentation than herbivores, due to their lower populations levels and their dependence on their prey (Denys & Tscharntke 2002;

Hunter 2002; Braschler *et al.* 2003; Schmidt *et al.* 2005, van Nouhuys 2005; Ewers & Didham 2006). Therefore, from the perspective of biological control, it is important to study whether changes in the agricultural landscape configuration, due to fragmentation and/or habitat loss, can affect the abundance of natural enemies, and their ability to maintain pests below economic injury levels.

Habitat loss and fragmentation usually occur simultaneously in nature, and for that reason they have been frequently confounded in the literature (McGarigal & Cushman 2002). But they are distinct processes, and their effects on biota can be quite different. Habitat fragmentation *per se* is the subdivision of an originally continuous habitat into more, smaller patches, and habitat loss is the removal of habitat, which might occur without fragmentation (Fahrig 2003). In studies where the effects of habitat fragmentation and habitat loss are isolated, often habitat loss has negative effects on the abundance and diversity of organisms, while fragmentation *per se* has no effect, or even may have positive effects on population abundance and species richness (Fahrig 2003; Grez *et al.* 2004a, 2004b, 2008; Zaviezo *et al.* 2006).

Here we show the results from two years of field experiments where we studied the effects of habitat loss and fragmentation on several predatory taxa (carabids, coccinellids and spiders) and aphids associated with alfalfa in Central Chile.

MATERIALS AND METHODS.

Experimental landscapes.

The field research was conducted at Antumapu Experimental Research Station, University of Chile, Santiago, Chile (33°34 ' S, 70°37 ' W), during the 2002–2003 and 2003–2004 growing seasons. For the experiments 30 × 30 m alfalfa (*Medicago sativa* L.) landscapes, separated by at least 20 m, were sown in August of each year. Landscapes were all originally continuous, but in December of each year, randomly selected landscapes were fragmented by plowing the alfalfa. In the first season, we evaluated the effects of habitat loss (0 or 84%), fragmentation (4 or 16 fragments) and isolation (2 or 6 m between fragments) on abundance and species richness, by creating five types of landscapes: unfragmented control (0% habitat loss), 4F – 2m (four 6 × 6 m fragments separated by 2 m, 84% loss), 4F – 6m (four 6 × 6 m fragments separated by 6 m, 84% loss), 16F – 2m (sixteen 3 × 3 m fragments separated by 2 m, 84% loss), 16F – 6m (sixteen 3 × 3 m fragments separated by 6 m, 84% loss) (Fig. 1a). In the second season, we evaluated the effects of varying levels of habitat loss (0, 55 or 84%) and fragmentation (4 or 16 fragments), maintaining fixed distance between fragments (6m), by creating four types of landscapes: unfragmented control (0% habitat loss), 4F – 55% (four 10 × 10 m fragments, 55% loss), 4F – 84% (four 6 × 6 m fragments, 84% loss), 16fr – 84% (sixteen 3 × 3 m fragments, 84% loss) (Fig. 1b). The matrix surrounding the alfalfa fragments was bare ground. We used a complete randomized block design, establishing fifteen landscapes distributed in three blocks for the 2002-2003 season, and twenty landscapes distributed in 5 blocks for the 2003-2004 season (Fig. 2). No insecticides were applied. For a detailed explanation of the crop management see Grez *et al.* (2004a, 2008).

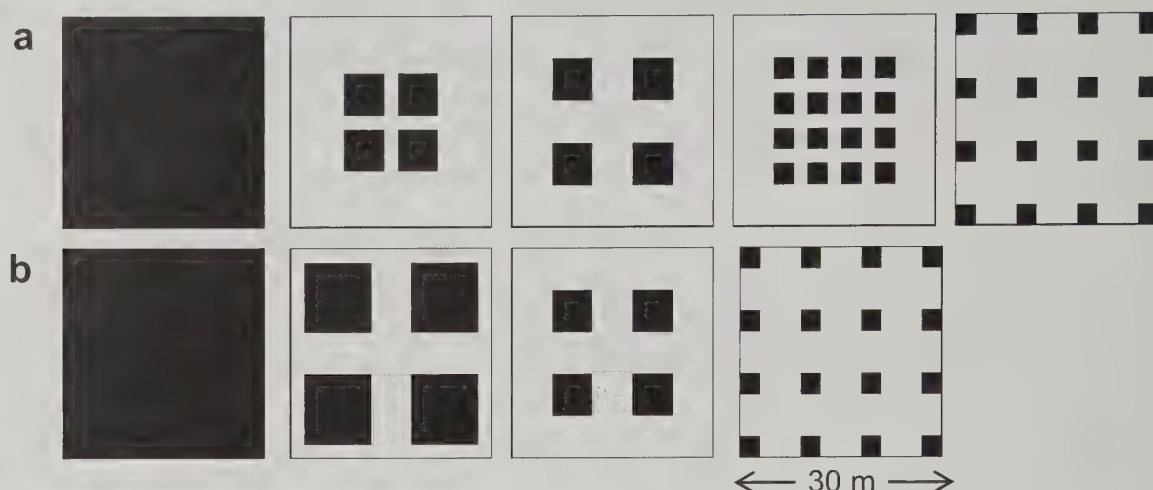


Fig. 1. Experimental landscapes established in 2002 (a) and 2003 (b). Each square represent a 30 x 30 m landscape, where the dark area stands for alfalfa and the white area for matrix (bare ground). In 2002 (a), landscapes corresponded to a control and four fragmented landscapes (84% habitat loss), created by the combination of two fragmentation levels (4 or 16 fragments) and two distances between fragments (2 or 6m). In 2003 (b) there was also a control, and three fragmented landscapes that varied in the degree of habitat loss (55 or 84%), fragmentation level (4 or 16 fragments) or both, with 6 m distance between fragments.

Insect sampling.

Carabids (Coleoptera: Carabidae) and spiders (Araneae) were sampled with pitfall traps, that consisted of transparent plastic containers (6 cm diameter and 8 cm depth), half-filled with a solution of water, formalin (10%), and detergent. We placed eight traps in each experimental landscape (one or two per fragment, and throughout the control landscape). Traps were kept open for four days on each sampling occasion and then brought to the laboratory to count and identify the specimens. Samples were taken in summer (1, 3, 5 and 7 weeks after fragmentation, December to February approximately), and in autumn, 13 and 15 weeks after fragmentation (March to April). On 2003-2004, an additional sample was taken 17 weeks after fragmentation. Spider data were analyzed only for the second season.

Adult coccinellids (Coleoptera: Coccinellidae) and aphids (Hemiptera: Aphididae) were sampled by sweep-netting. At sixteen (first season) or eight (second season) points in each experimental landscape, we took four sweeps with a 30 cm diameter net, covering a total area of approximately 4 m² of alfalfa. Samples were taken in summer and autumn in the same weeks when pitfall traps were set, but in the second season additional samples were taken 19 weeks after fragmentation for coccinellids and aphids, and 21 weeks after fragmentation for aphids (for exact dates see Grez *et al.* 2004a, 2004b and 2008). Additionally, on the 2002-2003 season, adult coccinellids in the whole area of 10 landscapes were sampled with sweep nets one and seven weeks after fragmentation, in order to study the spatial distribution of

the four most abundant species and their association in space (sampling details in Zaviezo *et al.* 2006).

Data analyses.

The effects of habitat loss, fragmentation, isolation or a combination of them (depending on the year) on density and species richness of coccinellids, carabids and spiders, and on the population abundance of aphids were analyzed for summer (short term) and autumn (long term) separately, since different demographic mechanisms may be more important in determining the abundance of the arthropods studied in different times of the year. For the analyses, we used the mean density of insects per landscape (averaging the sub-samples) to avoid pseudoreplication. Effects were studied through analyses of repeated measures ANOVA or covariance (ANCOVA) depending on the hypothesis, to be tested and times sampled in each period. Also, planned comparisons between means of different types of landscapes were carried out in order to test specific hypotheses.



Fig. 2. Aerial view of the experimental site (~5 ha), showing the distribution of the experimental landscapes in the field for the 2003-2004 season.

RESULTS.

Carabids.

In the first season, when we evaluated the effect of habitat fragmentation and isolation, with a 84% habitat loss, carabid abundance and species richness in the

short term (summer) were significantly higher in fragmented landscapes with higher fragmentation and isolation, i.e. 84% loss, 16 fragments, 6 m distance ($F_{4, 54} = 3.17$, $P = 0.02$, and $F_{4, 54} = 4.25$, $P = 0.005$ for abundance and species richness respectively, Fig 3A, C). In the second season, where landscapes varied in levels of habitat loss, fragmentation or both, carabid abundance and species richness was again higher in landscapes with highest habitat loss and fragmentation ($F_{3, 71} = 6.28$, $P = 0.001$ and $F_{3, 59} = 21.05$, $P < 0.001$ for abundance and species richness respectively, Fig 3B, D). In both years, these effects disappeared in the long term (autumn) ($P > 0.05$ for all), when populations increased in all landscapes to similar levels.

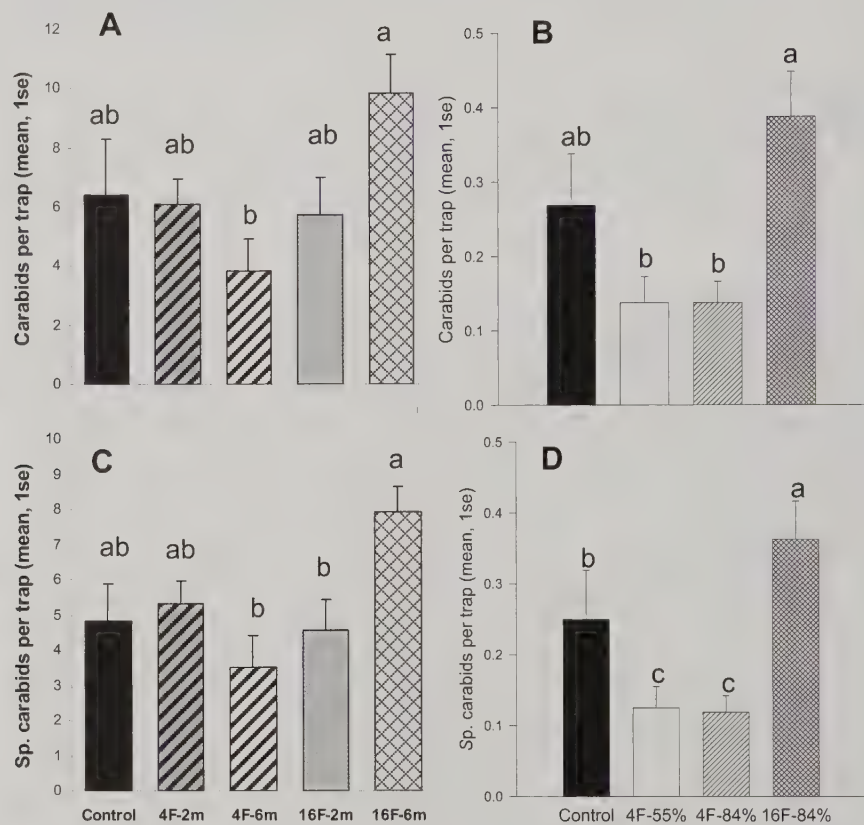


Fig. 3. Carabid abundance (top) and species richness (bottom) within experimental landscapes during the early sampling period (summer), for the 2002-2003 (A, C) and 2003-2004 (B, D) seasons. Different letters on the bars indicate significant differences after planned comparisons and Bonferroni correction.

Coccinellids.

In the first season, coccinellid total abundance did not differ between landscapes, but the four most abundant species were differentially affected by habitat loss, fragmentation and isolation. The abundance of the two exotic species *Hippodamia variegata* (Goeze) and *Hippodamia convergens* Guérin-Menenville in the short term (summer) were not affected by habitat loss ($F_{1, 11} = 0.03$, $P = 0.86$ and $F_{1, 11} < 0.01$, $P = 0.99$, respectively), but they were more abundant in the more fragmented landscapes, though only when separated by 2m (see Table 2 and Fig. 3 in Grez et al 2004a). On the other hand, the densities of the native species *Eriopis*

connexa (Germ.) and *Hyperaspis sphaeridiodes* Mulsant differed between landscapes. *Eriopis connexa* was negatively affected by habitat loss one week after fragmentation, but this reversed on week seven, with the unfragmented control having the lowest densities. *Hyperaspis sphaeridiodes* densities were positively affected by habitat loss in both sampling dates, being more abundant in fragmented landscapes than in the control. Among fragmented landscapes, both species were more abundant in more fragmented landscapes, i.e. 16 fragments, and additionally *H. sphaeridiodes* was positively affected by an increase in isolation distance (see Tables 1, 2 and Fig. 2 in Zaviezo *et al.* 2006). Samples taken in autumn showed that these effects disappeared in the long term.

In the second season, again total coccinellid abundance did not differ between landscapes, but at the species level variable effects were observed. In the short term *H. convergens* and *H. variegata* densities tended to be lower in landscapes with higher levels of habitat loss (84%), while *H. sphaeridiodes* was positively affected by habitat loss. These effects were lost in the long term, but in the case of *Adalia bipunctata* (L.), a species that is more abundant at this time of the year, abundances in landscapes with higher habitat loss were larger than for the control, specially in the more fragmented ones (see Table 1 and Fig. 5 in Grez *et al.* 2008).

Coccinellid species richness in the first season was positively affected by habitat loss and fragmentation, being higher in landscapes with 84% habitat loss and 16 fragments, independent of isolation distance, and lower in the unfragmented control ($F_{1, 4} = 14.55$, $P = 0.012$ and $F_{1, 4} = 37.34$, $P < 0.002$ for habitat loss and fragmentation respectively, Fig 4A). More over, the spatial analysis showed that species were not segregated in space, on the contrary in most landscapes they were positively aggregated, especially in landscapes with higher fragmentation and isolation distance (Zaviezo *et al.* 2006). In the second season, no significant effects on species richness were found in the short term ($F_{3, 59} = 1.21$, $P = 0.31$), but in the long term coccinellid species richness was larger in all fragmented landscapes than in the unfragmented control ($F_{3, 59} = 5.92$, $P = 0.001$, Fig 4B).

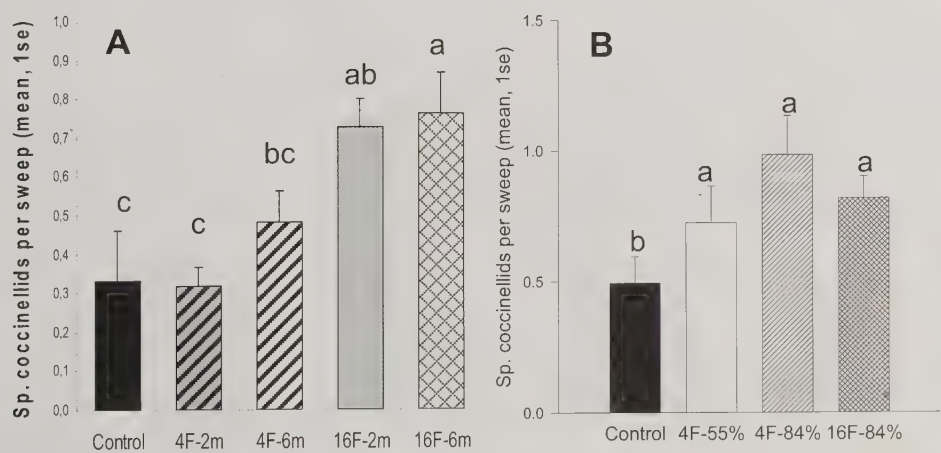


Fig. 4. Species richness (species per 4 m²) of coccinellids within experimental landscapes. For the 2002-2003 season (A) values are the mean for 1 and 7 weeks after fragmentation determined by intensive sampling. For the 2003-2004 season (B), values are for the mean of the late sampling period (autumn). Different letters on the bars indicate significant differences.

Spiders.

Spiders were analyzed only for the second season, with 19 species captured belonging to 11 families. The most abundant species were *Neomaso articeps* Millidge (Araneae: Linyphiidae), *Ostearius melanopygius* (O.P.- Cambridge) (Araneae: Linyphiidae), and *Lycosa indomita* Nicolet (Araneae: Lycosidae). Spider total abundance in the short term was similar for all landscapes ($F_{3, 59} = 2.00$, $P = 0.12$), but as in other predators sampled, there were significant effects at species level, with three of them showing higher abundances in the control. On the other hand, spider species richness at this time of the year was lower in some fragmented landscapes than in the control ($F_{3, 59} = 5.37$, $P = 0.002$), although it was similar among fragmented landscapes. In the long term, both total abundance and species richness were negatively affected by habitat loss, with the lowest values in landscapes with higher habitat loss (84% loss), regardless of their fragmentation level, and intermediate values in landscapes with 55% habitat loss ($F_{3, 39} = 12.68$, $P < 0.001$ and $F_{4, 39} = 4.09$, $P = 0.01$ for abundance and species richness, respectively). At this time of the year, spiders were more abundant and diverse in the unfragmented control landscapes.

Aphids.

In the first season, the most abundant aphid species in the study site was *Therioaphis trifolii* (Monell), with other species having very low abundances. No differences in aphid abundance among landscapes were found ($F_{4, 22} = 0.16$, $P = 0.96$). In the second season, *T. trifolii* was again the most abundant species followed by *Aphis craccivora* Koch. As for other groups, no differences among landscapes were found for total aphid abundance in the second season, but at the species level significant effects were detected, but only on the long term, when aphids were more abundant ($F_{3, 89} = 3.17$, $P = 0.03$ and $F_{3, 89} = 7.71$, $P < 0.001$ for *A. craccivora* and *T. trifolii*, respectively). While *T. trifolii* was more abundant in fragmented landscapes than in the control, *A. craccivora* showed the opposite trend (Fig 5 A y B, Grez et al. 2008).

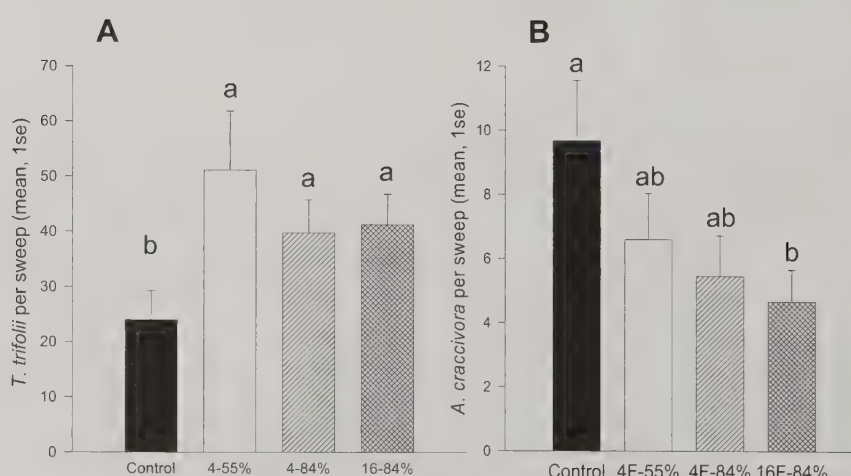


Fig. 5. Abundance of *Therioaphis trifolii* (A) and *Aphis craccivora* (B) within experimental landscapes for the 2003-2004 season during the late sampling period (autumn). Different letters on the bars indicate significant differences after planned comparisons and Bonferroni correction.

DISCUSSION.

After two years of field experiments studying the effects of habitat loss and fragmentation on arthropods associated with alfalfa some consistent effects were found. First, that the response to habitat loss and fragmentation was species specific, even within the same family or trophic level. Second, that many species were not significantly affected by habitat loss, fragmentation, isolation distance or a combination of the above. Third, that predatory species that were sensitive to landscape structure, were generally positively affected by habitat loss and/or fragmentation sometime during the season, with one notable exception: spiders, which were more abundant in unfragmented control landscapes. The effects of habitat loss and fragmentation were generally evident only at high levels, i.e. 84% habitat loss and 16 fragments. Fourth, isolation distance between fragments (tested in the first season), in general showed no effects on species abundance. Fifth, aphids also showed variable responses to habitat loss and fragmentation. These variable responses of species to landscape configuration may be explained by species dispersal behaviour (Tischendorf *et al.* 2005). Highly mobile species may be able to track more efficiently the landscapes discontinuities, and their densities should be less affected by habitat fragmentation than less mobile species (Ricketts, 2001; Ewers & Didham, 2006). This may explain why spiders are negatively affected by habitat loss and fragmentation while coccinellids and carabids, more mobile organisms, show the opposite trend.

Species richness of the different predatory taxa studied showed patterns similar to their abundance. Carabid and coccinellid diversity increased with fragmentation, habitat loss and isolation distance between fragments. This increase in species richness with increasing fragmentation has been also reported previously (Collinge & Forman, 1998; Tscharntke *et al.* 2002), suggesting that a landscape composed of a set of smaller and isolated fragments may support more species of insects than a larger and continuous landscape. Nevertheless, spider diversity was negatively affected by habitat loss and fragmentation.

In conclusion, our results suggest that high levels of habitat loss and fragmentation may affect the density and species richness of predatory insects, although the responses vary over time and are species specific, with some species being more sensitive to landscape configuration than others. These results should be considered when designing agricultural landscapes in order to enhance biological control.

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MORTALITY AND POPULATION DYNAMICS OF *BEMISIA TABACI* WITHIN A MULTI-CROP SYSTEM

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ABSTRACT.

The population dynamics of mobile polyphagous pests is governed by a complex set of interacting factors that involve multiple host-plants, seasonality, movement and demography. *Bemisia tabaci* is a multivoltine insect with no diapause that maintains population continuity by moving from one host to another over the year. To better understand the mechanisms governing population development of this insect within the landscape, whitefly "ecosystems" were established in three geographically and climatically distinct areas in Arizona. These systems consisted of a sequence of six representative hosts (winter broccoli, spring and fall cantaloupes, summer cotton, perennial alfalfa, various annual weeds, and the perennial ornamental, lantana). Source and rates of natural mortality were quantified, *in situ*, on each host plant by life tables. The quantitative contribution of each mortality factor varied among hosts and time of the year, but was relatively consistent among geographic sites. Predation (sucking insects) and dislodgement from the plant surface (largely chewing predation) were consistently the largest sources of mortality. Median generational mortality was generally > 90% except on spring-planted cantaloupe where median mortality was <70%. Low mortality during the spring on cantaloupe appears to act as a biotic release leading to outbreak populations during summer months. Overall, an understanding of the year round spatio-temporal dynamics of this pest and its associated natural enemies will greatly aid the development of better pest management strategies in all affected crops.

INTRODUCTION.

The concept that pest populations can be more efficiently managed at the landscape level has been viewed as a central, but little used, component of IPM for many decades (Rabb 1978; Kogan 1998). Such an approach is even more crucial to mobile, polyphagous pests which can readily exploit a wide range of both crop and non-crop hosts over time (Kennedy & Storer 2000). Implicit in this community perspective of pest management is the need for an understanding of and an ability to exploit natural enemies of the pest on an equivalent spatial scale (Landis & Menalled 1998; Schmidt *et al.* 2004).

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) is a pest of worldwide significance (Naranjo & Ellsworth 2001). The pest causes direct feeding damage, vectors over 110 plant viruses, induced plant disorders, and reduces yield quality through honeydew excretion. The pest potential

of *B. tabaci* is exacerbated by its polyphagy, high reproductive rate, dispersal ability and lack of a quiescent stage that enables year-round population development on a sequence of host plants in the landscape (Fig. 1).

The development of sustainable, ecologically-based management strategies for *B. tabaci* will depend on a mechanistic understanding of the complex, and interacting temporally and spatially-varying factors governing pest population development in a mosaic of hosts throughout the year. Various mortality agents including predation, parasitism, dislodgement from the plant surface, insecticides and physiological factors affect the survival of *B. tabaci* (Naranjo & Ellsworth 2005). An understanding of the timing, spatial distribution and magnitude of these mortality factors is central to their exploitation in pest management systems. Field-based life table studies were conducted over a three year period to quantify natural mortality and describe population change in multi-host systems in three regions of Arizona. Our goal was to compare and contrast the natural mortality factors affecting populations of *B. tabaci* at different times of the year to provide a mechanistic understanding of the pest's population dynamics and potential biological control in a landscape context.

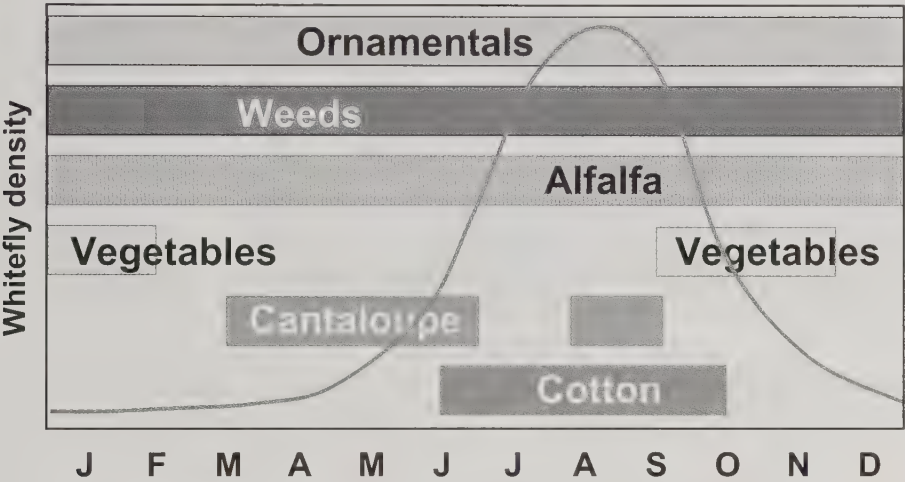


Fig. 1. A typical seasonal cycle of *B. tabaci* in a multi-host system in Arizona. Populations persist at low levels during winter months and may reach outbreak levels in summer in crops such as cotton.

MATERIALS AND METHODS.

Study sites were established in three geographically and climatically distinct areas in Arizona. In each area we established whitefly “ecosystems” consisting of a sequence of six representative hosts including winter broccoli, spring and fall cantaloupes, summer cotton, perennial alfalfa, various annual weeds, and the perennial ornamental, *Lantana*. No insecticides were used.

An *in situ* life table technique (Naranjo & Ellsworth 2005) was used to identify and quantify sources and levels of natural mortality affecting immature stages (eggs and nymphs) of *B. tabaci* on each host plant. Life table studies were conducted multiple times on each host plant throughout 3 years of study. Mortality was categorized as due to inviability (eggs only), dislodgement from the plant surface, parasitism, predation, desiccation, or other unknown factors (Fig. 2). Because multiple mortality factors act contemporaneously, stage-specific, marginal rates of

mortality were estimated for each factor based on apparent mortalities (Royama 1981; Elkinton *et al.* 1992; see Naranjo & Ellsworth 2005 for equations).

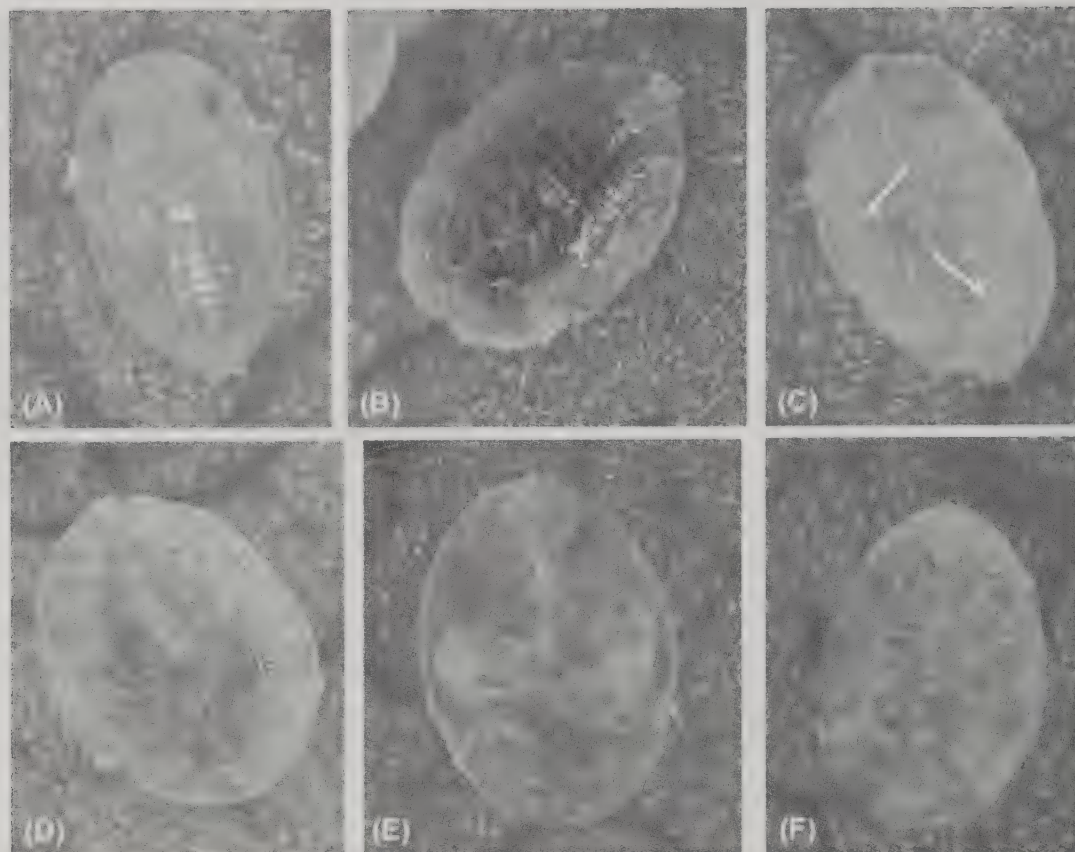


Fig. 2. Examples of natural-enemy induced mortality of *B. tabaci*; (A) *Eretmocerus* sp. parasitism, (B) *Encarsia* sp. parasitism, (C) early stage parasitism (displacement of mycetomes denoted by arrows), (D-F) predation by sucking predators.

RESULTS.

Generally, the same mortality factors were observed on all host plants; however, the relative contribution of each individual factor varied among host plants and the time of the year that they were grown (Fig. 3). Predation (primarily by sucking predators) and dislodgement (due to weather and chewing predators) were consistently the largest sources of mortality on all host plants except broccoli. Parasitism by aphelinids (*Eretmocerus* spp. and *Encarsia* spp.) was moderately high in alfalfa, lantana, fall cantaloupe and cotton (at the Maricopa site) and low on all other hosts. Egg inviability was consistently low throughout, but desiccation was highly variable across and within host plants depending on region. Desiccation was primarily associated with freezing temperatures that affected the insect or the host leaf it inhabited.

Generational mortality was variable but median mortality was > 91% in all hosts but spring-planted cantaloupes at the Maricopa and Yuma sites and fall cantaloupe at the Marana site where median mortality was <72%. Partitioned by season, predation and dislodgement were consistently the largest sources of mortality (not

shown). Parasitism was moderately high during the summer and fall, while desiccation was greatest during the fall and winter.

Mortality was not evenly distributed over the egg and four nymphal stages and in general, mortality was greatest during the 4th stadium, followed by mortality during the egg stage. The one exception was winter-grown broccoli in which considerable mortality also occurred during the 1st and 2nd nymphal stadia.

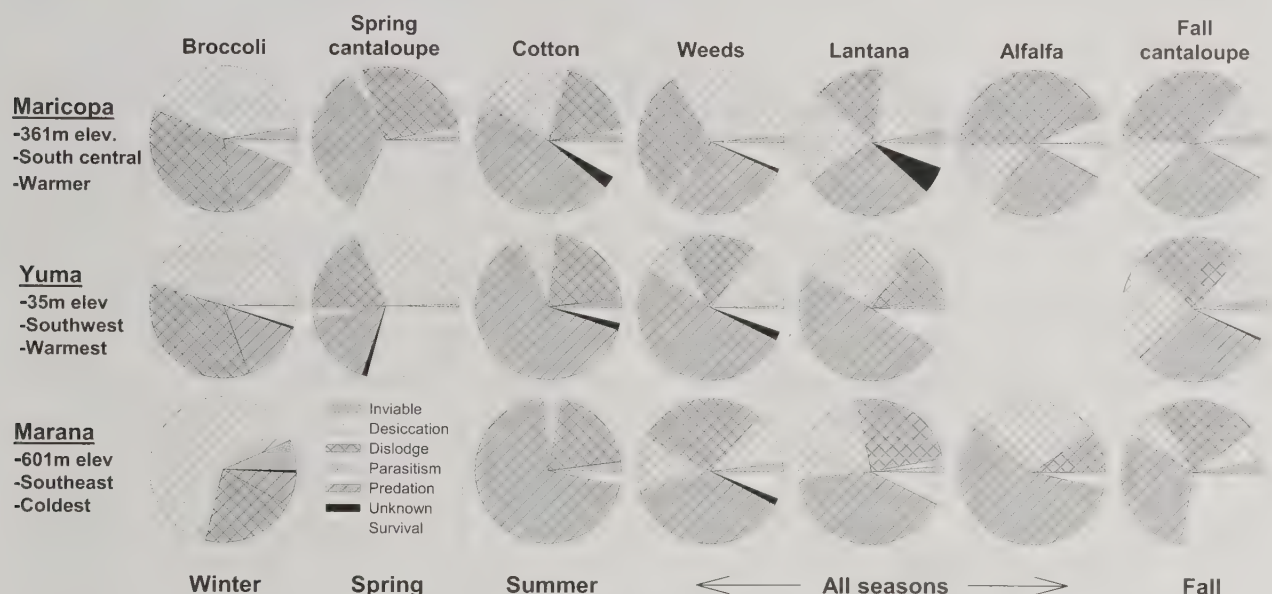


Fig. 3. Mean proportion (based on marginal mortality rates) of various mortality factors for *B. tabaci* populations on multiple host plants in three regions of Arizona, USA over a three year period. Summary based on 4-36 cohorts per host and site. Site descriptions indicate severity of winter months.

DISCUSSION.

Multiple mortality factors impact populations of *B. tabaci* and these factors along with host plant availability, dispersal and changing environmental factors govern the population dynamics of this pest insect throughout the entire year. Low populations during winter months are associated with high mortality from predation and weather related events such as dislodgement and desiccation from low temperatures along with reduced developmental and reproductive rates. Generational survivorship averaged < 9% on all host plants with the exception of spring-planted cantaloupes where survivorship averaged over 30%. Although parasitism was moderately high in these latter cases, predation was considerably lower than in others hosts and seasons. We have shown that predation is the key factor influencing variations in mortality of *B. tabaci* in the cotton system and perhaps the reduced level of this mortality factor in spring cantaloupes is associated with the lower mortality of *B. tabaci* on this crop. The high survival in the spring acts as a biological release that partial enables rapid population growth during the late spring and early summer leading to potential outbreak populations of *B. tabaci* by mid to late summer in Arizona that typically require insecticide applications for economic suppression. Better management of *B. tabaci* in spring crops such as cantaloupe may be key to population management of this pest within a region. Effort should be

placed on exploring options to increase biological control during this critical portion of the season.

Predation and dislodgement from the plant surface were consistently the largest sources of mortality across all hosts and sites during most of the season with the exception of desiccation from freezing temperatures which dominated in winter grown broccoli. Compared with previous life table studies in cotton (Naranjo & Ellsworth 2005), rates of parasitism were higher in cotton and in other hosts crops in our multi-crop landscapes. Aphelinid parasitoids do not appear to be strong dispersers (Hagler *et al.* 2002; Byrne & Bellamy 2003) and the close juxtaposition of host crops in this study may have facilitated higher parasitoids populations that were able to better track host resources and thus contribute more to pest mortality. This points to the potentially important role of crop diversification and/or that active management of habitats to enhance biological control by parasitoids (Landis & Menalled 1998; Gurr *et al.* 2004). The predators in this system are mostly generalist feeders that are well adapted to disturbance and readily disperse among host plants to take advantage of the changing abundance of prey populations. Nonetheless, active habitat management could potentially enhance the efficacy and impact of generalist predators as well, especially during the spring.

We have provided only a glimpse of our overall research effort that is focused on attempting to understand the complex dynamics of *B. tabaci* within a multi-host agricultural system in Arizona. Through a landscape lens, our results provide insight for improved management of all affected crops that take advantage of natural mortality forces such as natural enemies.

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THE IMPACT OF FLORAL RESOURCE SUBSIDIES AND LANDSCAPE COMPOSITION ON BIOLOGICAL CONTROL OF BRASSICA PESTS

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ABSTRACT.

Several studies have shown that complex landscapes with a high proportion of non-crop vegetation have higher densities of natural enemies and tend to have lower pest numbers than do simpler landscapes dominated by agriculture. However, it is not known how best to design landscapes to maximise biological control. Conservation biological control aims at improving the conditions for natural enemies of pests, for example by adding flowering plants that can provide non-prey food as well as shelter. To date, conservation biological control research has usually focused on plots or single fields while ignoring processes at larger spatial scales. However, it is likely that the effect of local habitat-manipulation measures will depend on the composition of the surrounding landscape. In the research project presented here we first investigate how vegetation composition at different spatial scales surrounding fields of forage brassicas in New Zealand determines biological control of the key pests diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and cabbage grey aphid, *Brevicoryne brassicae* (L.) (Homoptera: Aphididae). Second, we investigate how the effectiveness of local conservation biological control measures, in the form of floral resource subsidies is influenced by landscape features. Preliminary analyses show that parasitism and hyperparasitism rates of cabbage grey aphids and parasitism rates of diamondback moth increase with an increasing amount of non-crop vegetation in the landscape, whereas the type of non-crop vegetation appears to be of less importance. Further analyses are under way.

LANDSCAPE ECOLOGY OF *TRICHOGRAMMA* (HYMENOPTERA) IN NORTHERN NEW SOUTH WALES, AUSTRALIA

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ABSTRACT.

There is potential for using the wasp egg parasitoids, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), in Insect Pest Management programs for the control of Lepidoptera (moth) pests in Northern New South Wales, Australia. The objective of this project was to determine the landscape ecology of native and introduced *Trichogramma* spp. on the Liverpool Plains region to establish which habitats and combinations of crop and non-crop habitats contribute most to the parasitoid impacts on field crops. Parasitism levels were recorded in spring and summer crops, and all year round in native vegetation using eggs cards, natural egg collection, water traps and sticky traps. These were used to elucidate the population dynamics of *Trichogramma* spp. in habitat preferences, and species diversity. Current results indicate that summer crops such as unsprayed cotton and sorghum are good *Trichogramma* spp. habitats. Native vegetation is important in the overwintering of *Trichogramma* spp., particularly where native vegetation is not heavily grazed. Native vegetation, wheat and faba beans are the most favourable habitats in spring for *Trichogramma* spp., in comparison with lucerne, chickpeas, plainsgrass, and canola.

IMPACTS OF STAND SIMPLIFICATION OF VILLAGE FORESTS ON THE NATURAL ENEMY GUILDS OF PADDY FIELDS, AT SRINGERI TALUK (CHIKKAMAGALUR DIST., KARNATAKA)

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ABSTRACT.

This study was an attempt to understand the ecology of natural enemy guilds in a traditional upland paddy-agroecosystem with special references to the land-use changes of surrounding village forests. In this study we have addressed the following questions: (1) whether traditional uncultivated areas, called Soppina Bettas, with a higher vegetation diversity than other tree-based corridors can harbor more natural enemies during non-growing seasons; and (2). how Soppina Bettas adjacent to cultivated crops act as refuges for natural enemies of the pests during the uncultivated seasons. We used pitfall traps, malaise traps, sweep-nets, and all-out search methods for collecting insects. The natural enemies were removed from the samples, counted and identified to morpho-species with the help of expert taxonomists. Vegetation, environmental and microclimatic variables were also measured during the sampling period. Predatory insects showed high species richness and abundance in the paddy fields adjoining the corridors. Generalist predators like carabid beetles were more diverse in the fallow fields adjacent to Soppina Bettas, and least diverse in paddy fields adjacent to Australian wattle during the fallow period while spiders were more abundant in fallow fields close to Areca Gardens. Paddy fields in the proximity of Degraded Shrub and Soppina Bettas showed maximum species richness of predatory insects while it was least in the proximity of the Acacia plantations. Among major predators, ground beetle abundance showed a positive correlation with stand density of corridors. So keeping diversified corridors and the bunds (embankments or dikes), is crucial for conserving natural enemy populations in rice paddy agro-ecosystems. Insects collected from Acacia plantations showed high species turnover when compared to other tree based corridors. This could be due to the replacement of habitat specialists by recruiting generalist species which have a better chance of survival in monoculture plantations. The arthropod movement from adjacent uncultivated lands to the farmlands is more or less dependant on the dispersal capability, and is resource driven.

LANDSCAPE COMPLEXITY DIFFERENTIALLY BENEFITS FOURTH OVER THIRD TROPHIC LEVEL NATURAL ENEMIES

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ABSTRACT.

Theory predicts an increased sensitivity of higher trophic levels to changes in landscape structure associated with habitat loss. Natural enemies of important crop pests are known to benefit from landscape complexity, due to the presence of key resources, such as alternative hosts, in extra-field habitats. However, any benefits of increasing complexity may be counteracted if this also enhances enemies at the fourth trophic level. Differences in trophic specialization may further complicate these relationships. Remarkably, the influence of landscape structure on fourth trophic level enemies and their interactions is virtually unknown. We investigated the effects of landscape complexity on aphid-primary parasitoid-secondary parasitoid communities of a dominant crop, wheat, and a dominant extra-field weed, stinging nettle. Nettles have long been considered important extra-field reservoirs of aphid natural enemies in European agricultural landscapes. However, we found that primary parasitoid (3rd trophic level) communities attacking cereal aphids had little overlap with the communities attacking nettle aphids (22% shared species), while secondary parasitoids (4th trophic level) showed high levels of species overlap (73%) across habitats. Densities of primary parasitoids in wheat fields were unaffected, while secondary parasitoids tended to increase, with increasing landscape complexity. This resulted in a significant increase in rates of secondary (i.e., hyper) parasitism with increasing landscape complexity. Our results suggest that extra-field habitats in complex landscapes can differentially benefit fourth over third trophic level natural enemies. In cases where the fourth trophic level exerts significant impacts on the third, this would be predicted to disrupt biological control of crop pests.

MULTIPLE METHODS DEPLOYED TO UNDERSTAND THE EFFECTS OF LANDSCAPE STRUCTURE AND COMPOSITION ON NATURAL EMEMIES IN AUSTRALIAN AGRICULTURAL SYSTEMS

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ABSTRACT.

Increasingly, it is realised that the performance of natural enemies is affected by factors operating at scales beyond field - and even farm - boundaries. A range of methods is being applied and refined for understanding the temporal and spatial dynamics of natural enemy movement, direct and indirect effects on pest populations, and resulting levels of biological control. This paper presents results from three PhD projects, each supported by a different industry agency (for cotton, vegetable and farm forestry) where growers' funds are explicitly directed at understanding the effects of landscape structure and composition on biological control. Results will be reported from studies where arthropod movement has been tracked using dyes and rare earths, including a novel method where dispersal of rubidium-labeled *Trichogramma* (Hymenoptera: Trichogrammatidae) adults is monitored via detection of rubidium in the wasp progeny within host eggs. Arthropod data are integrated with landscape metrics and analysed at varying spatial scales to identify, for varying guilds, the habitat preferences and the spatial scale that most strongly determines within-crop numbers. Cost distance analysis further elucidates the ease with which natural enemies and pests move through landscape elements and reveals good scope to design landscapes that may selectively benefit natural enemy immigration into crops whilst deterring pests.

SESSION 8

RECENT ADVANCES IN CONSERVATION BIOLOGICAL CONTROL

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As more countries adopt active carbon trading in response to climate change, farmers will increasingly derive payment for sequestering atmospheric carbon. Whether that is done by tree plantings or boosting soil carbon with green manure crops, opportunities will present for biological control to be simultaneously promoted. A second factor leading to change in farming landscapes is the need to conserve biodiversity on farmlands; wildlife reserves are too scarce, too small and too dispersed to be the sole sites for effective conservation. This fact is likely to become a still more powerful policy driver, such that farmers' inherent tendency to make the places they live and work aesthetically pleasant will be complemented by 'carrot and stick' drivers imposed by governments. Thus, preservation and rehabilitation of native vegetation will be encouraged and this will bring exciting potential for farmers to 'grow their own' biological control agents. Of course, the growing body of international researchers concerned with habitat management will need to rise to the challenges of preventing enhanced vegetational diversity leading to additional resources for pest species. Researchers will also need to provide guidance on optimal spatial layouts for non-crop habitats such that they are effective sources of natural enemies that colonise crops sufficiently early in the growing season. Several of the themes introduced above are represented in the Habitat Management session at the 3rd International Symposium for Biological Control of Arthropods. The convenors received many offered papers and those selected to present talks constitute the tip of a large iceberg of active habitat management researchers. Speakers come from the UK, USA, New Zealand and Australasia and this illustrates the global interest in habitat management. The nexus between habitat management and biodiversity conservation is clear in two talks that explicitly investigate the utility of native and endemic plants to promote biological control. The other important research theme apparent in this session's talks is the wide range of scale, extending from chemical ecology of plant defences and natural enemy attraction to landscape level design. The overall aim of this session is to bring together key workers in habitat management and capture some of the excitement of working in a branch of biological control that is so intertwined with the wider forces influencing farming. We hope that all involved with the session (even those of us deeply immersed in the intricacies of chemical ecology or GIS data sets!) will better appreciate the holistic nature of our shared endeavour.

OVERVIEW, SETTING THE STAGE: HABITAT MANAGEMENT

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ABSTRACT.

Habitat management as a means to enhance the impact of arthropod natural enemies is an attractive alternative to inoculative and inundative forms of biological control. The risks associated with introduction of exotic agents are avoided as can be the costs and logistical complications of mass rearing and release of agents. Whilst habitat management-based biological control (aka conservation biological control and habitat manipulation) is not without its challenges, the level of interest in these methods amongst researchers, growers and policy makers continues to mount. Habitat management methods are being used in large-scale, commercial production systems in crops as diverse as wheat, lettuce, vineyards and citrus in the USA, Europe, Australasia and south-east Asia. Some of these strategies – alyssum strips within Californian lettuce for example - are ephemeral and tightly focussed on enhancing biological control but often the habitat management approach is a perennial groundcover or woody plant stand that provides a range of additional ecosystem services. This fact is important in policy frameworks promoting habitat management and in farmers adopting novel practices. For, when well-designed, natural enemy enhancement can be an ‘added benefit’ when manipulating farm vegetation for other purposes including catchment hydrology, erosion control, crop and livestock shelter, or aesthetics. This talk will explore the theme of biological control enhancement in the broader context of the changes to farming practice that are likely to result from climate change mitigation and the need to conserve biodiversity on farmlands.

PLANTS CRYING FOR HELP: HOW HIPPOS CAN IMPROVE CONSERVATION BIOLOGICAL CONTROL

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ABSTRACT.

Deployment of synthetic methyl salicylate (MeSA), a herbivore-induced plant volatile (HIPV), in controlled release dispensers (CRD) in hops and grapes in the Pacific Northwest (PNW) of the United States has been demonstrated to increase populations of beneficial insects, improve conservation biological control (CBC) and suppress pests. Other synthetic HIPV (e.g. hexenyl acetate, methyl jasmonate) have also been shown to attract beneficial insects in the field. Identifying and understanding the chemical-ecological and eco-physiological mechanisms mediating beneficial insect attraction to HIPV, is a current focus of this work. Determining whether beneficial insects are recruited by direct attraction or whether plants are stimulated by synthetic HIPV to produce their own beneficial insect-attracting volatiles, is a key question. Preliminary results from laboratory experiments indicate that hop plants exposed to CRD of MeSA emit a different profile of volatiles compared to unexposed plants. Some of these volatiles from MeSA-exposed plants are known attractants of beneficial insects and/or repellents for pests. Turning on or telling plants to produce their own natural enemy attractants is an appealing idea and strategy, and may be responsible for much of the success seen hitherto in improving natural enemy populations and CBC in PNW grapes and hops. Field experiments in Washington and New South Wales, Australia using agricultural spray oil formulations with MeSA or other synthetic HIPV, applied to hop, grape, sweet corn and broccoli plants, have demonstrated recruitment of some natural enemy species. This might be a more cost-effective and practical way of delivering HIPV or HIPPO (Herbivore-Induced Plant Protection Odours) to plants.

INTRODUCTION.

Attraction of insects to plants and other host organisms involves detection of specific semiochemicals (natural signal chemicals mediating changes in behaviour and development) or specific ratios of these semiochemicals (Pickett *et al.* 2006). Plants colonized and damaged by herbivorous insects produce a group of volatile organic compounds (VOCs) often referred to as Herbivore Induced Plant Volatiles (HIPVs), which may include semiochemicals that act as repellents for herbivorous pests and as attractants for organisms antagonistic to these pests, such as predators and parasitoids. In a dual-purpose role, these signals indicate that the plant is already infested and, therefore, less suitable as a host but they may also increase foraging by predators and parasitoids (Pickett *et al.* 2006). It is now clear and widely accepted that plants respond to attack by specific herbivore species, tailoring their induced direct and indirect defences accordingly (Karban & Baldwin 1997; Lou *et al.* 2006). Chemical defences that target the herbivore directly result in retarded development of the herbivore or death (Lou & Baldwin 2003), whereas indirect

defences increase herbivore mortality through the recruitment of parasitoids and predators with certain HIPVs (Thaler 1999; Kessler & Baldwin 2001). Studies on the mechanisms leading to the production of HIPVs have revealed the role of herbivore-specific elicitors (Mattiacci *et al.* 1995; Alborn *et al.* 1997; Halitschke *et al.* 2001). These elicitors can activate various signalling pathways in the plant resulting in accumulation or release of defence chemicals (Kessler & Baldwin 2002). Biological control agents (natural enemies) use a range of these VOCs to locate their prey.

Conservation biological control (CBC) seeks to preserve the resident natural enemy populations in a cropping setting, while enhancing their abundance and activity particularly through cultural techniques. Exploitation of chemical ecology in this approach involves incorporating practices that attract natural enemies into the cropping system while providing suitable nutrient sources within the system. Strengthening the natural enemy community both in terms of population density and species diversity is the aim of much of this research (Cardinale *et al.* 2003). Inevitably there are two aspects of this problem that need to be addressed: (1) attraction of beneficial arthropods to the crop during early cropping phases and (2) maintenance of these populations throughout the life of the crop. Manipulation of on-farm habitats to improve the attractiveness of crop ecosystems to beneficial arthropods is a major area of current research (Landis *et al.* 2000). This strategy is based on the idea that providing more and better resources (e.g. nectar, refugia) will allow larger populations of beneficial arthropods to reside in and near crops. Numerous examples of the potential and practicality of such approaches are available (Landis *et al.* 2000; Midega & Khan, 2003; Gurr *et al.* 2004; Midega *et al.* 2006; Koji *et al.* 2007). The use of semiochemical attractants (e.g. host/prey-derived chemicals) to increase recruitment and retention of beneficial arthropods in crop ecosystems is an area of opportunity for enhancement of CBC. Kean *et al.* (2003) identified 'spatial attraction' of natural enemies as the best way of enhancing CBC. Their results suggested an almost linear relationship between natural enemy attraction and prey equilibrium. The exploitation of semiochemical attractants in CBC to date is limited. However, research on semiochemicals and the natural enemies of herbivores has expanded greatly in recent years (Pickett *et al.* 2006).

HERBIVORE INDUCED PLANT PROTECTION ODOURS (HIPPOS).

Herbivore-induced plant volatiles (HIPVs) are a group of VOCs produced by plants specifically as a result of mechanical damage, through the feeding or oviposition behaviour of herbivorous pests. HIPVs have great potential for developing effective and practical semiochemical-based strategies for manipulating natural enemy populations in CBC. Based on the premise that synthetic HIPVs can aid CBC we refer to the latter as Herbivore-Induced Plant Protection Odours (HIPPOs). In essence, plants attacked by herbivores emit specific chemical signals. These are the 'words' of a complex language used to 'warn' other plants of impending attack and to recruit predatory/parasitic arthropods for 'bodyguard' services. Such plant 'bodyguards' respond to the language of plants in distress, and benefit from the food/host resources available (Sabelis *et al.* 1999). Extensive laboratory studies during the eighties and nineties, accelerating this century, in Europe, Japan and the US have investigated and unravelled many details and aspects of HIPVs relationships between plants and arthropods. HIPVs that attract predators and parasitoids include monoterpenes and sesquiterpenes from the isoprenoid pathway,

green leaf volatiles from the fatty acid/lipoxygenase pathway, products of the octadecanoid pathway, and aromatic metabolites of the shikimate, tryptophan and phenylalanoic ammonia lyase pathways (e.g. indole and methyl salicylate) (Pare & Tumlinson, 1996). The qualitative and quantitative characteristics of HIPVs can vary according to the herbivore involved, the plant species and even genotype (Turlings *et al.* 1993, Takabayashi *et al.* 1994). HIPVs may function as direct attractants and/or as plant signals. Topically applied methyl jasmonate (MeJA) (Hunter 2002) and the related cis-jasmone (Birkett *et al.* 2000) can act as plant signals by causing the emission of volatiles in some plants mimicking those produced in response to herbivore damage. There is also evidence that methyl salicylate and hexenyl acetate may also function as plant signals (Shulaev *et al.* 1997; Ozawa *et al.* 2000; Engelberth *et al.* 2004). The use of HIPVs as signals, elicitors or release primers of 'correct' and complete blends of natural enemy attracting emissions, is an attractive and tantalizing possibility for manipulating predator and parasitoid populations in pest management. However, compared to the abundance of laboratory studies on HIPVs, there is a dearth of field-based studies (Hunter 2002), that has only recently begun to be addressed. Until recently, no research had been reported on the use of synthetic versions of HIPVs as aids to either attract predators and parasitoids or to induce plants to produce their own HIPVs. Synthetic jasmonic acid applied directly to crop plants elicited production of HIPVs and increased parasitism of caterpillar pests (Thaler 1999). Kessler & Baldwin (2001) showed that synthetic HIPVs incorporated in lanolin paste applied near eggs of a moth increased predation by a predatory bug. The first demonstration of the potential of synthetic HIPV as direct field attractants for beneficial insects was provided by James (2003a, b, c). These studies showed attraction of a number of insect species and families to methyl salicylate (MeSA) and (Z)-3-hexenyl acetate (HA) in a Washington hop yard. Insects attracted to MeSA included the green lacewing, *Chrysopa nigricornis* Burmeister (Neuroptera: Chrysopidae), the bigeyed bug, *Geocoris pallens* Stal. (Hemiptera: Geocoridae), the mite-eating ladybeetle, *Stethorus punctum picipes* (Casey) (Coleoptera: Coccinellidae) and species of hoverflies (Diptera: Syrphidae). Three beneficial species were attracted to HA, a predatory mirid bug, *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae), a minute pirate bug, *Orius tristicolor* (White) (Hemiptera: Anthocoridae) and *S. punctum picipes*. Subsequent synthetic HIPV/trapping studies revealed at least 13 species or families of beneficial insects responded to one or more synthetic HIPVs. Thirteen HIPVs attracted one or more species/family of beneficial insect (James 2005).

HIPPOS IN ACTION.

Evidence for recruitment and retention of beneficial insects in grapes and hops in experiments in 2003 using controlled-release dispensers of MeSA, was presented in James & Price (2004). In a replicated experiment conducted in a juice grape vineyard, sticky cards in blocks baited with MeSA captured significantly greater numbers of five species of predatory insects (*C. nigricornis*, *Hemerobius* sp. (Order: Family), *D. brevis*, *S. punctum picipes*, *O. tristicolor*) than unbaited blocks. Four insect families (Syrphidae, Braconidae, Empididae, Sarcophagidae) were also significantly more abundant in the MeSA-baited blocks, as indicated by sticky card captures. Canopy shake samples and sticky card monitoring conducted in a MeSA-baited, unsprayed hop yard indicated development and maintenance of a beneficial arthropod population that was nearly four times greater than that present in an

unbaited reference yard. Four times as many *S. punctum picipes* and six times as many *O. tricolor* were sampled in the MeSA yard. Similar contrasts in abundance of these predators and others were apparent when compared with levels recorded in the yard in previous years. The large population of predatory insects in the MeSA-baited hop yard was associated with a dramatic reduction in spider mite and aphid numbers, the major arthropod pests of hops, in late June and sub-economic populations were maintained for the rest of the season. Further data on the effects of MeSA dispensers in enhancing CBC in hops and grapes were provided in James et al. (2005). The evidence presented in these papers and James & Price (2004) shows that the use of controlled-release MeSA in crops can potentially increase recruitment and residency of populations of certain beneficial insects improving CBC.

Field data for a possible plant signaling function of MeSA, MeJA and HA were obtained in a 2004 study conducted in a juice grape vineyard (James & Grasswitz, 2005). Blocks within the vineyard were exposed to dispensers of either MeJA, MeSA or HA. Beneficial insect populations were monitored using sticky yellow cards from April-September. Numbers of an encyrtid parasitoid of scale insects (*Metaphycus* sp. (Order: Family)) were higher throughout the season in the baited blocks than in the unbaited blocks. A similar effect was observed for *Anagrus* spp. (Order: Family) (egg parasitoids of grape leafhoppers) during late summer and autumn. These insects did not respond directly to MeSA, MeJA or HA (James 2005) and we hypothesize that they responded to an HIPV blend released by grape plants in response to exposure to MeJA, MeSA and HA. That is, the synthetic HIPV apparently 'signaled' to the plants an 'attack warning', resulting in the production of parasitoid-recruiting volatiles. Further field evidence for a plant-signaling function of MeSA and HA in eliciting indirect defense responses in plants was obtained in a more recent study. The abundance of some carnivorous and parasitic insects was significantly increased near hop and grape plants sprayed with botanical oil pesticides containing MeSA and HA (James *in press*). It seems likely that the plants were 'signaled' by MeSA, HA or other plant-derived compounds to emit a blend of volatiles to recruit 'bodyguards'. Exposure to MeSA and HA was likely interpreted by the plants as evidence of pest attack against nearby plants and a warning to defend themselves. Charleston *et al.* (2006) showed application of a botanical extract made from the syringa tree, *Melia azedarach* (Family), to cabbage plants, increased plant volatile emission and attracted the wasp parasitoid, *Cotesia plutellae* (Kurdjumov) (Order: Family). The 'signaling' compound in *M. azedarach* was not identified. These results provide further evidence of elicitor roles for MeSA and HA and provide optimism that these compounds can be used in crop protection programs to improve CBC.

The use of botanical oil based pesticides in combination with HIPVs like MeSA and HA, as a strategy for alerting plants to a herbivore threat and inducing natural defenses, appears to have great potential. In three field experiments conducted on hops and wine grapes, plants sprayed with a fixed plant oil, canola, or an essential oil, peppermint, rosemary, pesticides formulated with small concentrations of the HIPVs, MeSA or HA, attracted significantly greater numbers of some predatory and parasitic insect species, than unsprayed plants (James *in press*). Hop plant cultivar strongly influenced the results obtained in this study, suggesting that botanical oil/HIPV induced plant volatile emissions varied qualitatively and/or quantitatively

according to cultivar (Gouinguene *et al.* 2001; Lou *et al.* 2006). Similar experiments currently underway in New South Wales, Australia, using other HIPV as well as MeSA, on grapes, broccoli and sweet corn, are also providing good results (Simpson, Gurr, Simmons, James, Wratten and Leeson, unpub. data). These studies demonstrate the potential use of botanical oil based pesticides in combination with HIPV, as a strategy for alerting plants to a herbivore threat and inducing natural defences. Plants may respond by emitting blends of volatiles tailored to recruit natural enemies specific to herbivore pests of the emitting plant and improving the prospects of biological control. Herbivore-induced plant signals may also function as 'primers', alerting nearby plants to a potential herbivore or pathogen threat, but without invoking a full defense mechanism or strategy (Engelberth *et al.* 2004). Primed plants respond quicker to herbivore or pathogen attacks than unprimed plants. Artificially 'signaled' plants might also invoke direct biochemical defense mechanisms to decrease their palatability to herbivores and increase their resistance to pathogens (Arimura *et al.* 2000; Shulaev *et al.* 1997). Currently, the use of controlled release dispensers of MeSA is being extensively field-tested in hop yards and vineyards in Washington State and is showing considerable potential in increasing resident populations of beneficial insects and improving biological control (James 2003 a, b; James & Price 2004; James *et al.* 2005). The use of spray-applied MeSA in botanical oil pesticides may provide an alternative method of delivering the same result.

PROSPECTS FOR USING HIPPOS IN CONSERVATION BIOLOGICAL CONTROL.

The crop protection implications for using HIPVs as an aid to CBC are significant and exciting and hopefully further field-based research in other crop systems will yield results comparable to those obtained in hops and grapes. Similar examples exist where use of synthetic HIPVs or 'precursors' have resulted in reduced pest populations and enhanced natural enemy abundance and activity (Turlings & Ton 2006). Treatment of agricultural crops with either controlled-release dispensers or botanical oil based pesticides containing synthetic HIPVs to 'turn on' indirect plant defenses and enhance recruitment of natural enemies of pests, has the potential to be both effective and practical. However, numerous questions remain to be answered concerning aspects of the deployment and optimization of this strategy as a means of improving CBC, as well as possible undesirable ramifications. The 'signaling' hypothesis is at this stage the most plausible and reasonable explanation for the field successes seen in hops and grapes, but clearly requires validation, from well designed laboratory experiments. Analysis of the volatiles emitted by various crop plants exposed to botanical and/or mineral oil pesticides with or without MeSA or HA and the response of natural enemies to these volatiles, would be most instructive. Initial studies with hop plants indicate that exposure to MeSA for 72 hr results in increased emission of volatiles including MeSA and farnesene (James, Hebert, Wright and LePage, unpub. data). More study is also required on the influence of plant cultivars on HIPV emissions and natural enemy attraction and other possible influencing factors like fungal infection, nutrient availability etc (Gouinguene & Turlings 2002; Gouinguene *et al.* 2001). Research on the 'strength' of artificially applied 'signals' needed for priming plants to respond to herbivore threats, compared to the signals required for invocation of full defense, is also needed. Signaling crop plants to fully defend themselves when herbivores/pathogens are not present may

result in unacceptable costs to the plant if it adversely affects harvest yield/quality etc. It could also lead to specialist natural enemies learning to associate HIPVs with an absence of prey. Generalist natural enemies would perhaps be less likely to develop a negative association, if at least some prey type was present. The research published on synthetic HIPVs and natural enemy attraction in hops and grapes cannot be extrapolated to provide likely outcomes for their use in other crops. Exposure of other crop types to MeSA and HA may result in emission of different HIPV blends and consequent attraction of different natural enemies. Thus, the strategy needs to be researched and tailored to individual crop types and agricultural environments. The diversity and abundance of natural enemy communities surrounding crop ecosystems is also likely to have a substantial impact on the success of synthetic HIPVs in drawing predators and parasitoids into crops. Retention of natural enemies in crops could be improved by providing nutritional resources in the form of nectar-bearing ground covers, an approach which dovetails nicely with natural enemy recruitment by HIPVs (Attract and Reward). An alternative approach to using synthetic HIPVs to directly or indirectly attract beneficial insects is to genetically modify crop plants to enhance or modify HIPV production. The first steps on this path have recently been taken with genes controlling production of specific HIPVs successfully inserted into plants not normally able to produce these HIPVs. Transformed plants were then demonstrated to be attractive to predatory mites or parasitic wasps (Kappers et al. 2005; Schnee et al. 2006). Production of crop varieties that have improved abilities to attract natural enemies of herbivores is still some way off, but is an area that is likely to receive much attention in the future. For most natural enemies, the key attractants remain to be identified, so considerably more basic research on HIPV production by crop plants attacked by specific herbivores, is still required.

CONCLUSIONS.

Exploiting herbivore-induced plant volatiles to enhance the efficacy and reliability of CBC is a developing field, both exciting and rich in potential (Turlings & Ton 2006). The substantial data and literature resource accruing from basic and primarily laboratory-based studies over the past two to three decades on HIPVs, is providing an excellent foundation and framework for applied and ecological studies in the field. The prospect of using either airborne or spray-applied HIPVs to stimulate emission of HIPV blends from crop plants which mimic those emitted when the plants are attacked by pests, is particularly appealing. Whether this translates to increased recruitment of important natural enemies and enhanced CBC of crop pests, is a question that must be answered for each crop and geographic situation.

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CONSERVATION BIOLOGICAL CONTROL OF *RHOPALOSIPHUM PADI* IN SPRING CEREALS: WHAT INFLUENCES THE EFFICACY OF GENERALIST PREDATORS?

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ABSTRACT.

We have studied the impact of generalist natural enemies on the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphidae), in the cereal agroecosystem in Sweden for over 30 years. The complex of ground-living generalist enemies has been shown to be valuable for holding aphid infestations of the crop below the economic threshold. This presentation will highlight some of the factors that have been shown to be important for successful conservation biological control. We have used in-field and regional sampling methods to determine abundance and diversity of predators and prey. Modeling has also been used to study the influence of predator behavior on control efficacy. The influence of landscape and farm management has been considered. Economic impact of predators has been estimated. Various methods, including molecular methods, have been used to estimate the predatory potential of individual species. Ground living generalist enemies play a major role in conservation biological control (CBC) in spring cereals. Their impact is influenced both by local, field factors as well as landscape and farm management. High abundance of predators and synchrony with aphid infestation are essential for successful CBC. How this may be accomplished will be discussed.

THE INFLUENCE OF NATIVE PLANTS ON ARTHROPOD BIODIVERSITY AND CONSERVATION BIOLOGICAL CONTROL IN URBAN LANDSCAPES

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ABSTRACT.

Research has shown that predators and parasitoids of phytophagous arthropods suppress pest populations more often and successfully in the presence of a diversity of alternative prey species. This relationship forms a central tenet of conservation biological control and has stimulated efforts to attract sustainable populations of alternative prey to anthropogenic ecosystems. Plant-insect interaction theory predicts that specialist herbivores will be unable to grow and reproduce on alien plant species with which they have no evolutionary history. If this is so, the overuse of alien ornamentals in suburbia may have reduced the ability of native phytophagous insects to support the diverse population of natural enemies needed to control outbreaks of pest species. Our project tests the hypothesis that urban landscapes of native plants will sustain a greater diversity of native insect herbivores, and thus their natural enemies, than landscapes of alien plants. We predict that this complex of natural enemies will prevent pest outbreaks without the use of pesticides. We used simulated urban landscapes to compare the density and diversity of phytophagous insects and their natural enemies in plots comprised entirely of native or alien woody ornamental plants. We also compared survival of common herbivores and the time landscapes remained below their aesthetic injury level. These results demonstrate the potential for native plants to enhance conservation biological control efforts and provide a new tool for pest managers. Equally important, this study will establish baseline data on the importance of native plants in maintaining biodiversity in an expanding urban environment.

CONSERVATION OF NATURAL ENEMIES: STRATEGIC PLACEMENT OF A MULTIFUNCTIONAL HABITAT IN THE LANDSCAPE

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ABSTRACT.

Habitats placed in agricultural landscapes can have multiple functions including trapping insect pests, providing food and hosts for predators and native or exotic parasitoids of insect pests, conserving insect pollinators, and protecting wildlife. Stink bugs move through the landscape in search of food and oviposition hosts, and they exhibit a pronounced "edge effect" during these movements. Thus, a multifunctional habitat should be positioned between sources of stink bugs and a cash crop to exploit these stink bug behavioral tendencies. This paper includes one example of a multifunctional habitat in an agricultural farmscape. In the southeastern U.S., corn fields often are closely associated with cotton fields. In these corn-cotton farmscapes, *Nezara viridula* and *Euschistus servus* nymphs and adults disperse from corn into cotton at the interface, or common boundary of the two crops. A small habitat of milkweed was placed at the interface of a corn-cotton farmscape to determine the potential for this plant species to serve as a multifunctional habitat. The stink bug adult parasitoids, *Trichopoda pennipes* and *Cylindromyia* spp., and other pollinators, including honey bees, fed on nectar of milkweed. The stink bug egg parasitoids, *Trissolcus basalis* and *Telenomus podisi* also fed on nectar of these flowers. In addition, insecticides were never applied to the plants. Therefore, strategically placing a habitat of milkweed in the landscape can conserve insect pollinators and natural enemies of stink bugs.

INTRODUCTION.

Pollinators are essential for the reproduction of more than two-thirds of the world's crop species. Beneficial insects, both parasitoids and predators of pest insects, can play an important role in reducing or controlling populations of pest insects in agricultural farmscapes. Many of these beneficial insects and insect pollinators depend on nectar for their survival in these farmscapes. Laboratory and field cage studies demonstrate that parasitoids provided with sugar have greater longevity, fecundity, and more female biased sex ratios than starved parasitoids (Idris & Grafius 1995; Dyer & Landis 1996; Berndt *et al.* 2002). Parasitoids have been observed to feed on floral nectar in the field (Jervis *et al.* 1993). However the proportion of insects that utilize floral nectar in these diversified habitats is largely unknown.

Tropical milkweed, *Asclepias curassavica* L. (Asclepiadaceae), is often grown as a frost-tender annual in temperate areas of the U.S. The plant grows 0.6-0.9 m tall. The showy flowers are borne in terminal and axillary clusters, and they bloom continuously from spring to fall in temperate climates. The flower clusters are 5.1-

10.2 cm across with 6-15 flowers in each cluster. In addition to the normal calyx and corolla that other plant flowers have, milkweeds also have a corona that looks like an extra set of petals facing toward the center of the flower, like a crown. The corona is formed by five cuculli, and each cucullus consists of a cup-like hood that holds nectar.

Habitats of nectar-producing plants established in agricultural farmscapes could have multiple functions. In these farmscapes, it may be important to provide nectar as a food source to beneficial insects and insect pollinators especially at critical times during the season when nectar may not be available from other sources in the farmscape or when attempting to establish an imported parasitoid species. A beneficial habitat could also serve as an insecticide-free zone to conserve beneficial insects and bees and other insect pollinators. Generally, in the southeastern U. S. corn fields are closely associated with cotton fields. In these corn-cotton farmscapes, some *Nezara viridula* (L.) and *Euschistus servus* (Say) (Heteroptera: Pentatomidae) nymphs and adults that develop in corn disperse into cotton where they aggregate while feeding on cotton bolls. Therefore, there is a pronounced edge effect observed in the distribution of stink bugs as they colonize the new crop, cotton. Thus, these multifunctional habitats must be strategically placed in these farmscapes to exploit these stink bug behavioral tendencies. Because the flowers of milkweed provide a rich supply of nectar, and they bloom continuously from spring through the fall in temperate zones, establishing a habitat of tropical milkweed could possibly enhance beneficial insects and pollinators in south Georgia farmscapes. Thus, the purpose of this particular study was to monitor feeding activity of these insects on nectar of tropical milkweed in a corn-cotton farmscape in south Georgia.

MATERIALS AND METHODS.

Eight potted plants of flowering tropical milkweed were placed 1 m apart in a line parallel to the outside row of a corn field in Mystic, GA. The “Silky Gold” cultivar with yellow flowers was used. Insects visiting these milkweed plants were observed weekly throughout the corn growing season in 2008. Each plant was observed for 2 min. on an hourly basis from 09h00 to 17h00. Every known insect that fed on nectar of these plants during this observation period was recorded in the field. Small parasitoids and any unknown insects that fed on milkweed nectar were collected and brought into the laboratory for identification to family, genus, or species. Voucher specimens of all insects are held in the USDA-ARS, Crop Protection & Management Research Laboratory in Tifton, GA. Feeding behavior of 12 *Trichopoda pennipes* (Fab.) females and 14 *Cylindromyia* sp. (Diptera: Tachinidae) females was observed and recorded as these stink bug adult parasitoids fed on nectar of the tropical milkweed. Mean number of nectar-feeding insects per plant for each observation day and over the time of day were obtained for lady beetles, *Orius insidiosus* (Say) (Hemiptera: Anthracoridae), free-living wasps and flies, bees, and stink bug egg and adult parasitoids using PROC MEANS (SAS Institute 2003).

RESULTS AND DISCUSSION.

The tropical milkweed plants flowered throughout the corn growing season, and many species of beneficial insects and insect pollinators fed on nectar in flowers of

the plants (Table 1). This is the first detailed report of insects feeding on nectar of tropical milkweed, but families, genera, or species of many of the bees, free-living flies and wasps, and adult parasitic flies in the Tachinidae family previously have been reported to feed on nectar and pollinate other species of milkweed and other flowering plants. In general, less research has been conducted on *Euthera tentatrix* Loew (Diptera: Tachinidae) than on the other two stink bug adult parasitoids, *T. pennipes* and *Cylindromyia* sp., which may explain why there are no references of the former species on milkweed flowers. Only 5 *E. tentatrix* individuals were observed during this study and none were carrying milkweed pollinia.

Regarding adult parasitic wasps, only individuals in the Ichneumonidae family have been reported to feed on and pollinate milkweed (Robertson 1929). So this is the first record of scelionids and other small parasitoids feeding on nectar of any milkweed species. The braconid *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae) is a solitary endoparasitoid of the tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), and can contribute substantially to the biological control of its host (Tillman 2006). This parasitoid readily fed on nectar of tropical milkweed flowers. The scelionids, *Trissolcus basalis* (Wollaston), *Telenomus podisi* (Ashmead), and *Telenomus* sp. (Hymenoptera: Scelionidae), the encyrtid *Ooencyrtus* sp., (Hymenoptera: Encyrtidae) and the eupelmid *Anastatus* sp. (Hymenoptera: Eupelmidae) are parasitoids of stink bug eggs (Jones 1988). These tiny parasitoids fed on nectar usually near the side of the flower between two cuculli probably in order to avoid drowning in nectar.

Adults of four species of Coccinellidae were observed feeding on nectar of tropical milkweed flowers (Table 1). These predators are commonly found preying on a wide variety of prey in corn (Quaintance & Brues 1905). Most insects that feed on nectar of milkweed flowers in turn pollinate the flowers. However, a reference for lady beetles pollinating milkweed was not found, and none of the many lady beetle individuals observed on milkweed plants in this study were found with pollinia on their bodies. Apparently, lady beetles take nectar from milkweed flowers without providing any pollination service. The predator *O. insidiosus* is an abundant natural enemy of *H. zea* in various cropping systems including corn (Quaintance & Brues 1905). Like the tiny wasp parasitoids, many adults of this predator were located near the side of the flower between two cuculli and appeared to be feeding on nectar, but some individuals were also observed feeding on a thrips species in the flowers. A more detailed study on the feeding behavior of this predator on milkweed needs to be conducted in the laboratory.

Table 1. Species of insects feeding on nectar of tropical milkweed in southern Georgia and associated references.

Insect Group	Family	Species	Milkweed nectar feeder	Milkweed pollinator	General pollinator
Bees	Apidae	<i>Apis mellifera</i> L. <i>Bombus</i> spp. <i>Xylocopa virginica</i> (L.) <i>Megachile</i> sp. <i>Toxomerus marginatus</i> (Say) <i>Rivellia</i> sp. <i>Musca domestica</i> L. <i>Phytocephala</i> sp. <i>Polistes carolina</i> (L.) <i>Polistes fuscatus</i> (F.) <i>Cerceris</i> sp. <i>Scolia nobilitata</i> F. <i>Campsomeris plumipes fossilana</i> (F.) <i>Prionyx parkeri</i> Bohart and Menke <i>Sphex ichneumoneus</i> (L.) <i>Myzinum maculata</i> (F.) <i>Myzinum</i> sp.	species ^{1,2,3,4} genus ^{1,2,3,4} species ³ genus ^{1,2,4} species ⁴ genus ⁵ family ⁴ genus ⁴ genus ¹ species ^{2,4} genus ^{3,4} genus ⁴ species ⁴ genus ^{1,2,4} species ^{1,2,3,4} genus ^{1,2,3,4}	species ^{1,2,3,4} genus ^{1,2,3,4} species ³ genus ^{1,2,4} family ⁶ family ⁴ genus ⁴ genus ¹ species ^{2,4} genus ^{3,4} genus ⁴ species ⁴ genus ^{1,2,4} species ^{1,2,3,4} genus ^{1,2,3,4}	species ⁷ genus ⁷ genus ⁷ family, genus ⁷ family, species ⁷ family ⁷ family, genus ⁷ family, genus ⁷ family, genus ⁷ family, genus ⁷ family ⁷ species ⁷ family, species ⁷
Free-living flies	Megachilidae Syrphidae Platystomidae Muscidae Conopidae Vespidae				
Free-living wasps	Crabronidae Scoliidae Sphecidae Tiphidae				
Parasitic flies	Tachinidae	<i>Trichopoda pennipes</i> (F.) <i>Cylindromyia</i> sp. <i>Euthera tentatrix</i> Loew	genus ⁴ genus ⁴	genus ⁴ genus ⁴	
Parasitic wasps	Scellionidae Encyrtidae Eupelmidae Braconidae Coccinellidae	<i>Trissolcus basalis</i> (Wollaston) <i>Telenomus podisi</i> Ashmead <i>Ooencyrtus</i> sp. <i>Anastatus</i> sp. <i>Cardiochiles nigriceps</i> Viereck <i>Hippodamia convergens</i> Guérin-Ménéville <i>Coccinella septempunctata</i> (L.) <i>Coleomegilla maculata</i> (De Geer) <i>Harmonia axyridis</i> (Pallas) <i>Orius insidiosus</i> (Say)	family ¹ family ¹ family ¹ family ¹		
Lady beetles					
Other predators	Anthroconidae				

REFERENCES: ¹Eastman 2003; ²Betz *et al.* 1994; ³Kephart & Theiss 2008; ⁴Robertson 1929; ⁵MacPherson 2008; ⁶Wigney 2008; ⁷Moisset 2006.

The seasonal occurrence of lady beetles, *O. insidiosus*, free-living flies and wasps, bees, and stink bug egg and adult parasitoids feeding on nectar of tropical milkweed is shown in Fig. 1. The predators, including the four lady beetle species and *O. insidiosus*, were relatively the most abundant insects visiting milkweed flowers in May and early June. A second peak of lady beetles occurred the end of July. At this time, lady beetles apparently moved out of dying corn onto milkweed plants where they stayed for only a short period of time. Stink bug egg parasitoids were observed feeding on milkweed nectar early in the season which coincided with the time in which stink bug eggs were observed in corn. The number of free-living flies feeding on milkweed nectar began to build up the end of May and increase through the middle of June. Free-living wasps and bees fed on nectar of tropical milkweed throughout the season, but they began increasing in mid-to-late June and were relatively the most abundant insects visiting milkweed flowers the later half of the season. Stink bug adult parasitoids were observed feeding on milkweed nectar from early June through the rest of the season the same time adult stink bugs were observed feeding and/or mating in corn.

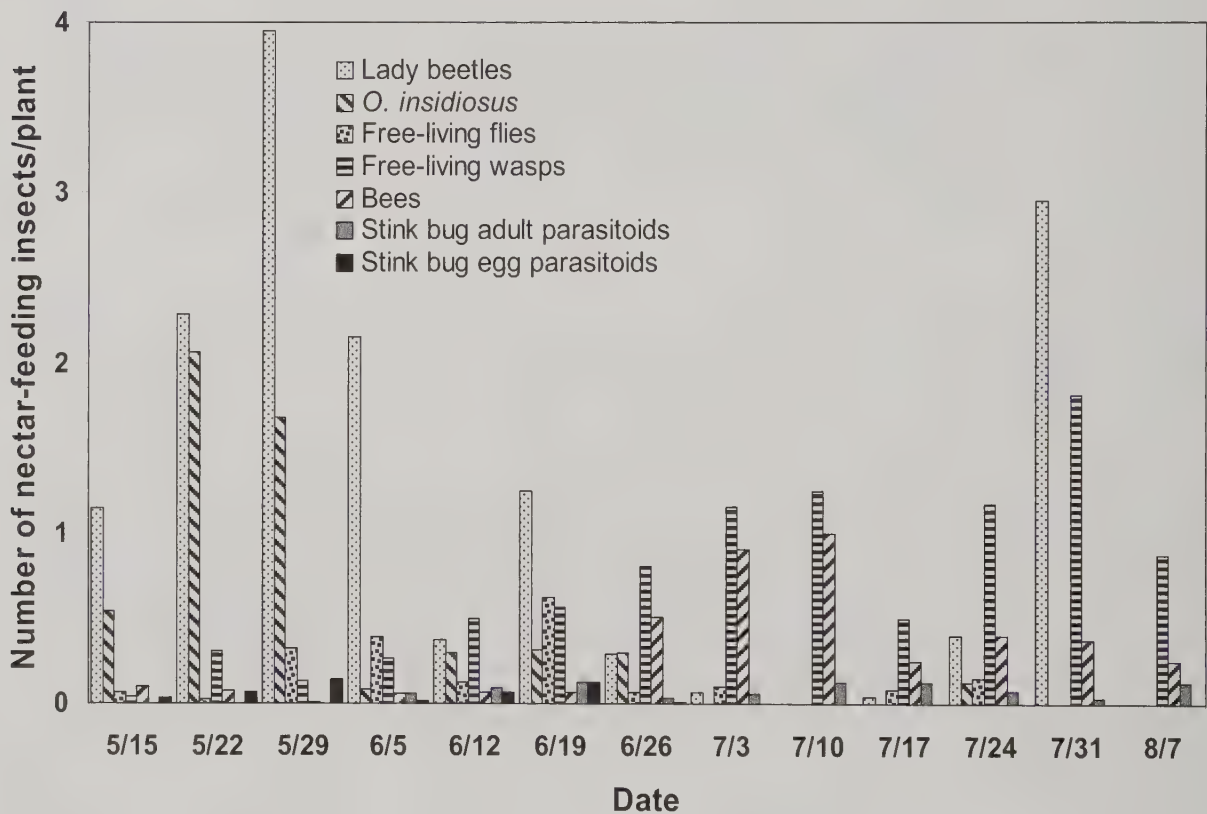


Fig. 1. Mean number of nectar-feeding insects per tropical milkweed plant over the growing season in Mystic, Georgia in 2008.

Because the mean number of lady beetles, *O. insidiosus*, free-living flies, and stink bug egg parasitoids feeding per milkweed plant was relatively high on 29 May, the diurnal feeding activity of these insects was examined on this day (Fig. 2). Ladybeetles were most active from 11h00 through 17h00. Adults of *O. insidiosus* appeared to be equally active throughout the day. Free-living flies and stink bug egg parasitoids fed on milkweed nectar from 10h00 to 16h00. The mean number of free-living wasps, bees, and stink bug adult parasitoids was relatively high on 3 July, and so the diurnal activity of these insects was examined on this day (Fig. 3). Bees were

most actively feeding on nectar from 11h00 to 12h00. Free-living wasps were most actively feeding on nectar at 13h00. Stink bug adult parasitoids were observed feeding on milkweed nectar from 10h00 to 13h00. Examining overall activity of insects feeding on tropical milkweed nectar, it appears that the best time to observe nectar-feeding activity of these insects would be from 10h00 to 14h00 during the summer months.

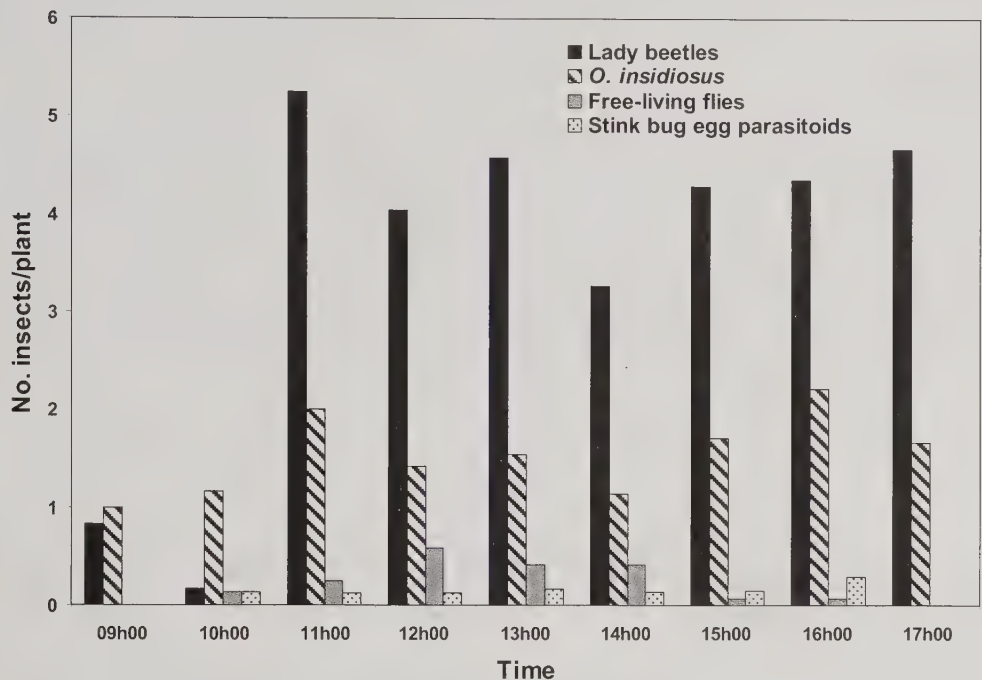


Fig. 2. Mean number of nectar-feeding insects per tropical milkweed plant from 09h00 to 17h00 on 29 May 2008 in Mystic, Georgia.

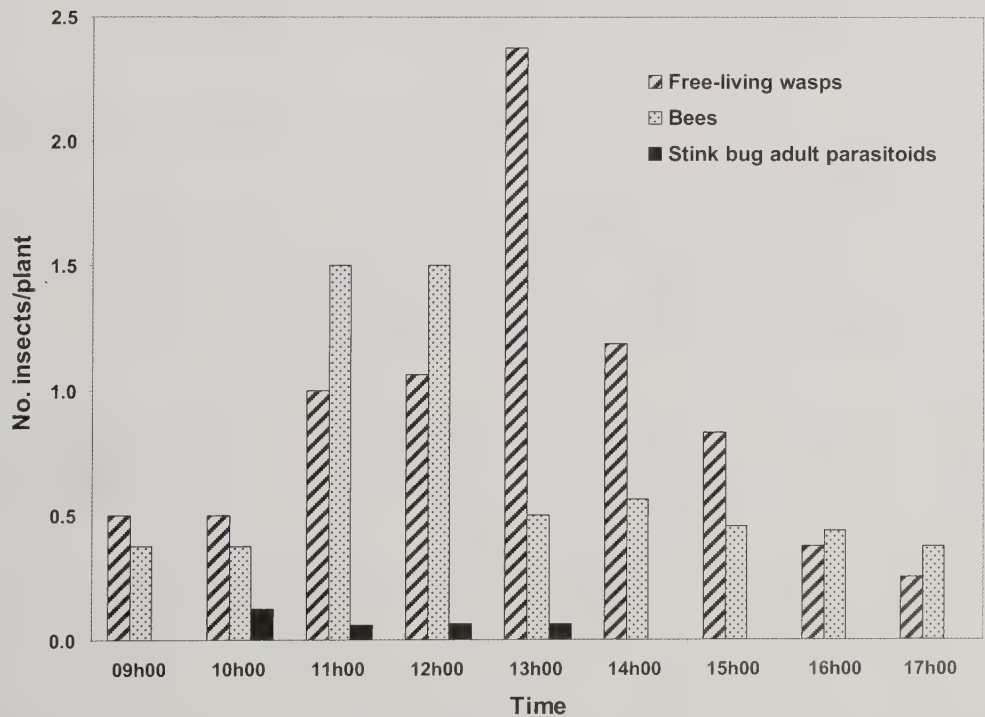


Fig. 3. Mean number of nectar-feeding insects per tropical milkweed plant from 09h00 to 17h00 on 3 July 2008 in Mystic, Georgia.

Some feeding behavior of *T. pennipes* and *Cylindromyia* sp. was observed as these stink bug adult parasitoids fed on nectar of tropical milkweed. For the 12 *T. pennipes* females observed, a range of 1-13 flowers were fed on during the time the females were searching for food in the milkweed habitat, and feeding time per flower ranged from 3-35 sec. For the 14 *Cylindromyia* sp. females observed, a range of 1-16 flowers were fed on when females were searching for food in the habitat, and feeding time per flower ranged from 3-18 sec.

CONCLUSIONS.

The flowers of tropical milkweed are attractive to beneficial insects and insect pollinators, and they provide nectar to these insects on-farm in south Georgia. Corn is a host plant of stink bugs, and several predators and parasitoids were observed attacking the various developmental stages of this pest in this crop. Corn plants do not produce nectar, and so an addition of a habitat of nectar-producing tropical milkweed plants in this environment can conserve beneficial insects and insect pollinators.

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ENDEMIC NEW ZEALAND PLANTS FOR PEST MANAGEMENT IN VINEYARDS

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ABSTRACT.

The use of endemic plants to provide both ecological and economic value to viticulture and to agriculture in general is a relatively new endeavour. Few studies have assessed the specific contributions that endemic plant species may make towards ecosystem services such as conservation, weed suppression, soil stabilisation, carbon sequestration, biological control etc. within heavily modified agricultural settings. Such services have, however, undergone extensive study with the conclusion that they are vital to maintain and improve the productivity of agricultural systems. Consequently, endemic plants that can provide ecosystem services have the potential to be of significant economic and ecological value.

The enhancement of ecosystem services, such as biological control, is one way in which endemic flowering plants may add value to agricultural production. Utilizing endemic, rather than non-endemic, flowering plants in and around agricultural systems provides potential not only for enhanced pest control, but also for conservation, ecosystem restoration and an enhancement of landscape complexity, reducing risk associated with monocultures. New Zealand is home to over 2,500 native plant species of which over 80% are endemic and rare in agricultural areas. This firstly means some form of screening is required to select which of these 2,500 species are suitable to provide floral resources within the agricultural setting, and secondly that by propagating these species we are contributing to regional restoration and conserving global biodiversity.

This paper presents preliminary investigations into how nectar 'sugar signatures' of these plants might be used as a means to shortlist potential endemic floral resources for enhancing parasitoid fitness and discusses how current research aims to assess the contribution of native plants deployed in a New Zealand vineyard to pest management.

INTRODUCTION.

Within most countries the vast majority of lowland habitat has been converted to agricultural production and additional conversion is highly likely to follow as world demand for food increases (Scherr & McNeely 2008). Modern agriculture with its intensive production systems is widely acknowledged as the primary cause of biodiversity loss on the planet (Matson 1997). An estimated 4973 million hectares of the Earth's land surface have been converted to agriculture (FAOSTAT 2003). Today, however, there exists an opportunity for agriculture to become a key contributor to biodiversity conservation, and itself benefit. This outcome is possible if growers become aware of the production benefits of biodiversity upon their land, and are thus motivated to actively restore or protect that biodiversity. For instance the simplification of an agricultural landscape increases the risk of pest outbreaks due to

the homogenization of the cropping system. This results from monocultures being unable to support sufficient populations of natural enemies due to a lack of food resources and overwintering sites (Corbett & Rosenheim 1996). By establishing or maintaining non-crop vegetation as habitat for natural enemies, pest damage and the costs for controlling pests could thus be reduced.

The viticultural industry of New Zealand is rapidly expanding in its area of cultivation, with 2006 seeing a 12% increase, bringing the total vineyard area to 24,721ha and some regions seeing growth of up to 53% (New.Zealand.Winegrowers, 2007). Similar to the situation in California there is concern that there exists little pest suppression by natural enemies due to a lack of natural habitats within often monocultural vineyards (Nicholls *et al.* 2008). However, current viticultural expansion within New Zealand is by no means causing significant natural habitat loss; this occurred decades ago with initial human settlement. Rather, today's new vineyards are typically replacing highly modified pastoral land. However due to consumer and market demands for more sustainable production systems and 'green' products (i.e., fewer or no pesticide applications), the viticulture industry is in a position where restoration of habitats for natural enemies could prove beneficial for production and profit, including eco-tourism, as well as conservation benefits.

'Ecoagriculture' or 'agroecology' is a concept whereby agricultural production can benefit from conserving or enhancing farm biodiversity (Scherr & McNeely 2008). An initiative in North Canterbury New Zealand called 'Greening Waipara' exemplifies the adoption of this concept (see <http://bioprotection.org.nz/greening-waipara>) where by grape growers are establishing native plants within and around their vineyards for production *and* conservation benefits. These benefits, perhaps more correctly termed 'ecosystem services', include conservation biological control. This approach uses habitat management to create a farm setting that is favourable to natural enemies and or unfavourable to crop pests (blue book). Non-crop plants grown around or within the crop can benefit natural enemies by providing resources including shelter, nectar, alternative prey and or pollen (Landis *et al.* 2000) and result in greater biological control activity while reducing pesticide usage (Pimentel 1997; Wratten & van Emden 1995). So far research within New Zealand vineyards has shown that production costs can be reduced due to the need for fewer pesticide applications when the non-native plant buckwheat, *Fagopyrum esculentum* Moench (Polygonaceae) is sown within the vineyard (Berndt *et al.* 2006). If native plants can be found to offer similar pest control enhancement not only would production benefit but so too would conservation. The challenge then is to select native plant resources that have the potential to provide conservation biological control services and also to prove that having endemic plants within the vineyard does indeed improve pest control. The following sections discuss how current research at Lincoln University New Zealand is addressing these two challenges.

SELECTING ENDEMIC FLORAL RESOURCES TO ENHANCE PARASITOID FITNESS.

Seven flowering annual plant species are commonly recommended for use in agriculture for the purpose of enhancing pest control. These plants, including buckwheat, *F. esculentum*, alyssum, *Lobularia maritima* (L.) Desv. (Brassicaceae), mustard, *Sinapis alba* L. (Brassicaceae), faba bean, *Vicia faba* L. (Fabaceae), dill, *Anethum graveolens* L. (Apiaceae), phacelia, *Phacelia tanacetifolia* Benth.

(Hydrophyllaceae) and coriander, *Coriandrum sativum* L. (Apiaceae), have been shown to achieve some success at improving biological control, from improving parasitoid fitness in the laboratory (Baggen & Gurr 1998; Baggen *et al.* 1999; Irvin & Hoddle 2007; Irvin *et al.* 2006; Manojlovic *et al.* 2001; Vattala *et al.* 2006) to reducing pest damage in the field (Berndt *et al.* 2006; English-Loeb *et al.* 2003; Irvin *et al.* 1999; Lee *et al.* 2006). However, many of these plants are annuals and are deployed outside of their native range (Fiedler *et al.* 2008). If plants native to an area can be found to convey similar fitness benefits to natural enemies as these non-native plants then there exists the opportunity for biodiversity conservation and agricultural production to mutually benefit.

Because there are over 2,500 native New Zealand plants criteria are needed to shortlist potential candidates. Research to date has equipped us with several criteria that may be used to assess the suitability of a plant for use as a floral resource for parasitoids within agriculture. For instance, the resource must be attractive and accessible to the parasitoid (Baggen *et al.* 1999; Bugg & Van Horn 1998; Vattala *et al.* 2006; Wackers 2004), produce significant quantities of nectar or pollen (Zhao *et al.* 1992) and selectively enhance the 'fitness' of the natural enemy rather than the pest (Araj *et al.* 2008; Begum *et al.* 2006; Lavandero *et al.* 2006).

Another possible criterion to select a floral resource is nectar quality. Increasing the concentration of sugar solutions fed to wasps increases longevity while the chemical composition of nectar, which differs between flowering plant species, may influence parasitoid 'fitness'. Nectar composition is dominated by sucrose, glucose and fructose, all of which are suitable carbohydrate sources for parasitoids; however it has been suggested that the ratio of these sugars (sucrose/(fructose + glucose)) is important to parasitoid fitness (Baker & Baker 1983; Vattala *et al.* 2006). The potential thus exists for such 'sugar signatures' to be used as a criterion to shortlist potential floral resources for enhancing parasitoid fitness.

Although nectar qualities of a plant species vary with age, condition, contamination and various other abiotic and biotic plant factors (Azzouz *et al.* 2004; Burquez & Corbet 1991; Koptur 2005) robust testing by Baker and Baker (1983) showed that angiosperms could be assigned a nectar sugar ratio class. These four ratio classes were: sucrose-dominant (>0.99), sucrose-rich ($0.5-0.99$), hexose-rich ($0.1-0.499$) and hexose-dominant (<0.1). It was suggested that parasitoids would benefit most from sucrose-dominant nectar.

Research at Lincoln University has undertaken analysis of several native and non-native plant nectars with results from this work presented in Figure 1. HPLC analysis revealed the nectar ratio to significantly ($F_{(13,55)} = 53.3$, $P < 0.001$) differ between plant species, providing the opportunity to shortlist native plant floral resources according to parasitoid sugar ratio preferences. Current research is now clarifying whether the sugar ratio affects parasitoid fitness and so confirm if these nectar signatures can be used as a selection criterion.

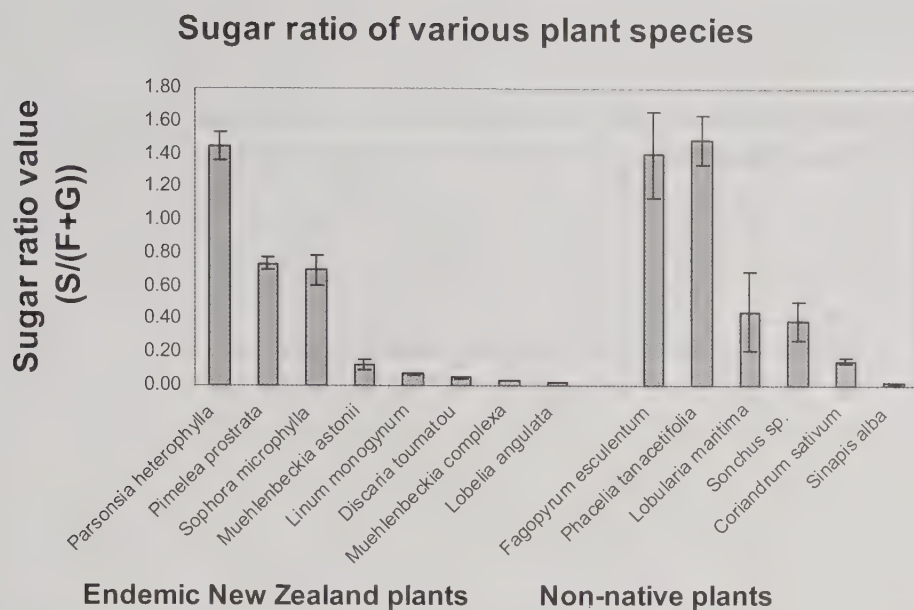


Fig. 1. The sugar ratios of various plant species determined through HPLC analysis of nectar. These ratios could consequently be used as a means to identify a potentially suitable floral resource for parasitoids.

Demonstrating that floral nectar of a certain quality enhances parasitoid fitness does not automatically mean it is suitable in all cropping situations and certainly not for all parasitoid species. Numerous factors determine whether a floral resource is to be successful at enhancing biological control in the field and that it will be adopted by growers. Orre *et al.* (2007) gave an in-depth review of considerations including the agronomic practicalities such as cost, plant availability, labour requirements and technology uptake by growers. Certain aspects of the floral resource need to be considered with respect to the parasitoid targeted for fitness enhancement. The resource needs to be selective (Lavandero *et al.* 2006) to ensure pest species do not benefit while plant flowering duration and timing, as well as accessibility and attractiveness (colour, shape, volatile cues) of the floral resource is important to the success of this pest management technique (Olson & Wackers 2007; Vattala *et al.* 2006; Wackers 2004; Wäckers 1994, 2005).

Consequently once it is known what optimal sugar qualities exist for certain parasitoid species there will still be several other important factors to consider in determining an optimal floral resource for a production system. However it is hoped that further clarification of parameters for plant selection, such as nectar quality, will aid in the identification and use of floral resources. Considering the vast potential for use of angiosperms other than those seven most commonly used any additional selection parameters will be of great help to create shortlists of suitable floral resources.

THE CONTRIBUTION OF ENDEMIC PLANTS TO PEST CONTROL IN VINEYARDS.

A typical New Zealand vineyard consists of row upon row of *Vitis vinifera* L. ssp. (Vitaceae) grapevine with a bare earth or shortly mown rye grass floor. Such an environment may be described as a simplified agri-ecosystem or indeed monoculture. Biodiversity provides several ecological services including the suppression of pest organisms (Altieri 1999), simplified systems host fewer natural enemies than complex systems containing a higher area of non-crop vegetation (Tscharntke *et al.* 2007). Many scientists have concluded that non-crop habitat is important for conserving natural enemies of crop pests (Altieri 1999; Fry 1995; Gurr *et al.* 2003; Landis *et al.* 2000; Ponti *et al.* 2005; Pywell *et al.* 2004; Sotherton 1984; Thies & Tscharntke 1999; Thomas & Marshall 1999), inferring that natural enemies are able to increase in number within these unsprayed refuges and then move out into the crop to provide pest control services.

Research within vineyards looking at biodiversity enhancement for ecologically-based pest control has found that non-crop refuges do lead to increased levels of pest control in adjacent vine rows (Nicholls *et al.* 2008) supporting the belief that increased agroecosystem diversity leads to greater pest control (Gurr *et al.* 2004). Non-crop habitats which are throughout the crop, such as a cover crop, have the potential to provide more effective pest control compared to corridors or field margins which may only enhance pest control within adjacent rows rather than throughout the entire vineyard. For instance Corbett & Rosenheim (1996) found that although prune trees, *Prunus domestica* L. (Rosaceae), near vineyards maintained overwintering populations of the parasitoid wasp *Anagrus epos* Girault (Hymenoptera: Mymaridae) and resulted in enhanced control of a vineyard pest, leafhoppers, this control was limited to only a few rows downwind. This demonstrates the importance of taking into consideration the spatial scale of habitat management for pest control, which must account for the dispersal capabilities of natural enemies targeted for enhancement (Landis *et al.* 2000) and also highlights a limitation to non-crop refuges which only border crops. Even cover crops, spaced throughout a crop, are eventually ploughed under, removing for a time this refuge for natural enemies and potentially negatively reducing their abundance and composition (Schellhorn *et al.* 2008; Sharley *et al.* 2008; Thorbek & Bilde 2004).

In order to provide a continuous and undisturbed refuge throughout a vineyard it is proposed that permanent groundcovers be established directly underneath the vines. Being permanent such groundcovers may offer critical overwintering sites for natural enemies that would otherwise vacate the vineyard system in the colder months when dormant vines and bare earth provide little or no suitable habitat. Such groundcovers would also potentially provide a raft of additional services such as erosion control, devigoration of unwanted vine growth, weed suppression, aesthetics, eco-tourism and help regulate soil moisture, organic carbon and biological activity. If native to the area the groundcover would contribute to the conservation of the plant itself as well as cultural values (Fiedler *et al.* 2008). The addition of these groundcovers will create a more diverse system, which in itself will improve the sustainability and health of the cropping system (Locke 2001).

Research at Lincoln University, New Zealand, is currently assessing the effects of native plant groundcovers upon invertebrate diversity and natural enemy abundance within a North Canterbury vineyard. A key vineyard pest within New Zealand is the light brown apple moth, *Eppiphyas postvittana* Walker (Lepidoptera: Tortricidae) which as larvae cause damage to both foliage and fruit. This pest also increases the incidence of grape bunch rot, caused by *Botrytis cinerea* (Persoon) Fries (Hyphomycetes), as it creates infection sites for the disease to enter (Lo & Murrell 2000). Natural invertebrate enemies of this pest include a range of parasitic wasps and flies, spiders, predatory bugs and mites and earwigs, *Forficula auricularia* L. (Dermaptera: Forficulidae). Predation is thought to be of major importance in limiting light brown apple moth populations accounting for 48-94% mortality (HortNet 2000).

A trial consisting of fourteen endemic New Zealand plant species was established beneath Pinot noir vines in October 2007 within a replicated block design consisting of ten replicates. Treatments thus included the fourteen endemic plant species, a control treatment of bare earth and a treatment of naturally occurring vineyard floor vegetation predominantly consisting of rye grass, *Lolium perenne* L. (Poaceae). In August 2008 treatments were sampled using a vortis insect suction sampling system as described by Arnold (1994). Samples were obtained with the vortis set on maximum suction for duration of 10 seconds and subsequently stored in 70% ethanol before being returned to the laboratory for sorting and identification of taxa. Individuals were then assigned an RTU for statistical analysis for diversity.

A total of 264 invertebrates from 11 taxa were collected including Araneae (72 individuals), Diplopoda (54), Diptera (37), Psocoptera (33), Hymenoptera (27), Hemiptera (20), Coleoptera (14), Neuroptera (3), Chilopoda (2), Acarina (1) and Pulmonata (1). Due to non-normally distributed data a non-parametric Kruskal-Wallis one-way ANOVA was undertaken which compared invertebrate diversity and richness between treatments. The Shannon index was used to ascertain diversity as this index takes into account both evenness and species richness (Magurran 1988). For diversity there were significant treatment effects ($H_{15} = 44.25$, $P < 0.001$) (Fig. 2). Using a Tukey pairwise comparison it was found that the endemic plant species *Geranium sessiliflorum* Simpson and Thomson (Geraniaceae), *Hebe chathamica* Cockayne et Allan (Plantaginaceae), *Anaphalioides bellidioides* Glenney (Asteraceae) and *Acaena inermis purpurea* Hook.f. (Rosaceae) as a group had higher invertebrate diversity than that of other plant treatments, bare earth or rye grass treatments (Fig. 2). *G. sessiliflorum* had the highest invertebrate diversity, which along with *H. chathamica* had a significantly higher diversity index than the control or grass treatments.

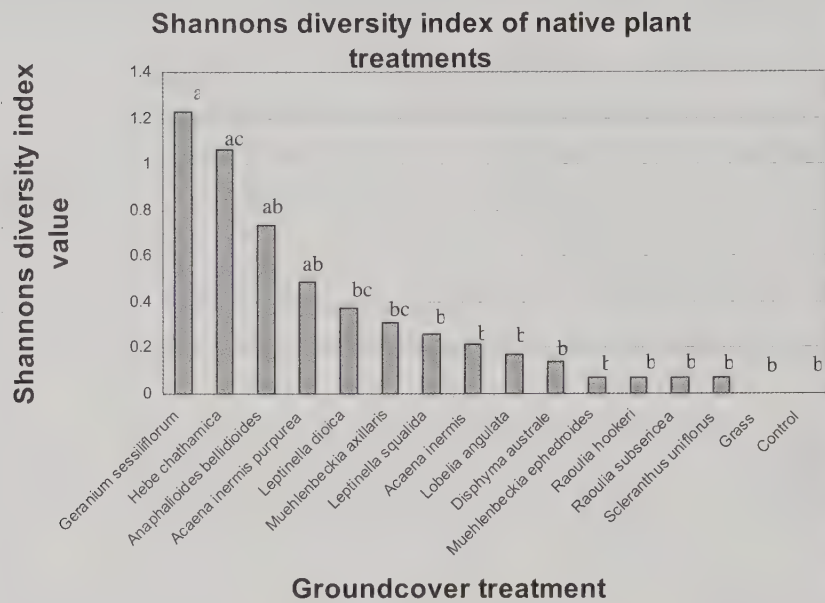


Fig. 2. Shannon diversity index value of different groundcover treatments. Different letters indicate values that significantly differ using a Tukey test $\alpha=0.05$. *G. sessiliflorum* and *H. chathamica* had a significantly higher diversity index value than grass or control treatments ($P<0.001$). Treatments which share letters do not significantly differ.

Spiders, which are key predators of the light brown apple moth larvae (Wearing *et al.* 1991) were the most abundant order of invertebrate to be sampled. Significantly more Araneae compared to the bare earth control and rye grass were found in plant treatments *G. sessiliflorum* and *H. chathamica* ($P<0.05$). A generalised linear model with a negative binomial error distribution was used due to non-normally distributed data. Of the plant treatments *G. sessiliflorum*, *H. chathamica*, *Muehlenbeckia axillaris* (Hook. F.) Walp. (Polygonaceae) and *A. bellidioides* had significantly more Araneae than the grass or control treatments ($\chi^2_{(2,15)}=26.36$; $P=0.034$) (Fig. 3). Spiders from the Araneae order included members of the Theridiidae and Clubionidae families which have been recorded as predators of the lightbrown apple moth in Canterbury (HortNet 2000).

Results so far show that certain species of native groundcover can support a greater diversity and abundance of invertebrate fauna than rye or bare earth in the winter season. However only two of the native plant groundcover species achieved this, indicating not all groundcovers will result in increased natural enemy abundance or invertebrate diversity. Reasons are likely to include the plants structural characteristics, size, microclimate and plant volatiles.

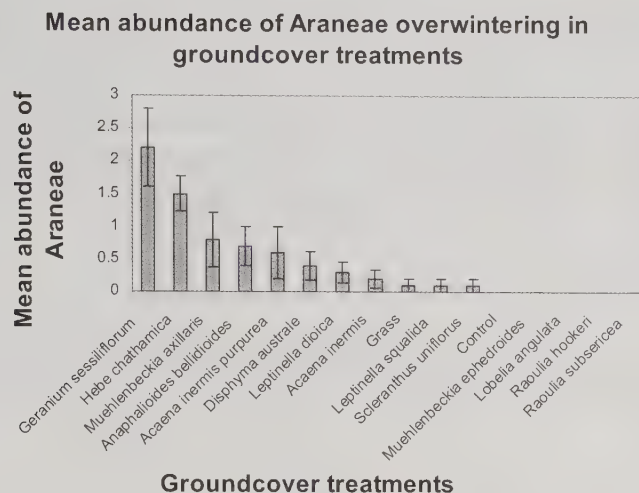


Fig. 3. Mean abundance of individual Araneae overwintering in groundcover treatments.

Although few numbers of invertebrate overall were found further assessment of the natural enemy diversity and abundance over the coming summer months is expected to provide greater clarification on how permanent native groundcover refuges contribute to pest control within the vineyard. Eventually the aim is to identify the native plants which encourage the desired biodiversity components for vineyard pest management. This would include not only natural enemies of the pest but also alternative hosts and resources for those natural enemies.

CONCLUSIONS.

With over 200,000 flowering plant species on the planet there is large scope for utilizing floral resources other than the seven most commonly used. Selecting floral resources native to a region will have further implications for improving biodiversity conservation within simple agricultural landscapes, potentially reversing the association of modern agriculture with biodiversity loss to an association of widespread biodiversity enhancement. Understanding more about what plant characteristics enhance parasitoid fitness would aid in the identification of potential floral resource species. Clarifying whether parasitoid fitness is optimised a specific nectar sugar ratio would provide an additional criteria for plant selection.

The establishment of native groundcovers beneath vines provides permanent non-crop refuges for a diversity of invertebrates including natural enemies of vineyard pests. The greater diversity of organisms found within native plant treatments compared to grass or bare earth treatments has implications for the resilience of the cropping system against pest outbreaks.

It would appear groundcovers allow natural enemies to maintain a presence in the vineyard over the winter period and as a consequence may enable an early population build-up of natural enemies at the beginning of the growing season which is then able to control pests earlier than if coming from refuges outside of the cropping area. This would have flow on benefits to the grower including reduced pest control costs, higher yields and a more sustainable cropping system due to

reduced reliance on external inputs such as pesticides. Future summer sampling during higher invertebrate activity is hoped to provide a larger data set from which to draw further conclusions regarding the use of these plants not only by natural enemies but also by the pest *E. postvittana*. Identifying which plant species selectively enhance natural enemies and are less attractive to pest species will also be paramount in determining plant species to recommend to growers.

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THE FUTURE OF HABITAT MANAGEMENT – UNDERSTANDING THE ROLE OF LANDSCAPE STRUCTURE IN THE PROVISIONING OF BENEFICIAL ARTHROPODS AND THEIR SERVICES

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ABSTRACT.

Within the context of conservation biological control, habitat management seeks to maximize natural enemy impact by providing limiting resources such as pollen, nectar, alternate hosts, or shelter. Past research on habitat management has primarily focused on maximizing the abundance and temporal duration of such local resources by establishing plant communities. These communities range from non-native monocultures of annual plants to perennial native plant polycultures. The influence of establishing resource plants on biocontrol services is variable, with the practice enhancing pest management in some, but not all cases. While several studies have examined the influence of resource plants on biocontrol services in adjacent croplands, few have considered the landscape context within which these habitats are located. Landscape structure has been shown to dramatically influence the diversity, abundance and impact of natural enemies within croplands. The ability of the landscape to supply insects to respond to habitat management is likely to greatly change the interactive effects of arthropod community structure, resource supply, and the realized ecosystem service of pest suppression. Therefore, to fully realize the benefits of habitat management the species pool of beneficial insects supported by a landscape must be considered.

KEYNOTE ADDRESS

AGROECOLOGY: CONTRIBUTIONS TOWARDS A RENEWED ECOLOGICAL FOUNDATION FOR BIOLOGICAL PEST CONTROL SYSTEMS

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ABSTRACT.

The quest for “silver bullet” products to control pest outbreaks has not been foreign to biological control, especially in augmentative approaches where the emphasis is placed on purchased biological inputs such as massive releases of parasitoids or purchased microbial pesticides widely applied in place of chemical insecticides. This strategy pertains to a dominant technical approach called *input substitution* where the thrust is to overcome at any given moment a factor limiting yield (insect pest) by using an appropriate external input (i.e. parasitoid release or microbial insecticide) thus perpetuating a process of treating symptoms rather than dealing with the ecological root causes of pest problems in modern agriculture.

It is herein argued that long term solutions to pest problems can be only achieved by restructuring and managing agricultural systems in ways that maximize the array of “built-in” preventive strengths, including the enhancement of functional biodiversity, with input substitution tactics serving strictly as backups of natural regulatory processes. Emergent ecological properties develop in diversified agroecosystems allowing biodiversity to thrive and establish complex food webs and interactions that lead to pest population regulation. But biodiversification must be accompanied by improvement of soil quality, as the link between healthy soils and healthy plants is fundamental to truly ecologically based pest management. Results from several studies, including results from our research on broccoli based cropping systems and vineyards provide interesting evidence to support the view that the long-term joint management of plant diversity and soil organic matter can lead to better plant resistance against insect pests.

SESSION 9

OMNIVORY IN BIOLOGICAL CONTROL

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Natural enemies, particularly predators, commonly engage in omnivorous feeding; and theoretical and experimental studies demonstrate the potential for profound effects of this feeding strategy on the outcomes of biological control. Omnivorous feeding is generally defined in two ways: either as feeding at two trophic levels within a food web (Pimm & Lawton 1974), or more narrowly, as feeding on both plants and animal prey (Lincoln *et al.* 2004; Coll & Guershon 2002). The consequences for biological control, of predators feeding on both herbivores, and other natural enemies can be predicted in the context of community-based predator-prey interactions. Predators that feed on both plants and prey, however, have complex relationships with plants and exhibit numerous biochemical, physiological, anatomical and behavioral trade-offs to allow them to feed on these two, very disparate resources. Given this complexity, the consequences of feeding on both plants and animal prey might be expected to be idiosyncratic to each plant-omnivore combination, and to defy prediction in the context of biological control. Feeding on plant resources by predators is important in many aspects of biological control – conservation biological control, and insectary plants being two examples. It is therefore important to understand the role plants and plant communities play in biological control in a general and predictive way. We know that omnivores feed on plant resources and they get benefit from it but, which are the priorities of omnivores when selecting their habitats? Do omnivores select plants that provide food resources by themselves and then feed opportunistically on the prey that happen to be on these plants? Or, do they look for prey and take advantage of the resources plants provide? Or both!? Do plants provide the clue where to look for prey and the subsistence fuel for omnivores in their quest for prey in an uncertain environment? Our purpose in organizing this session was to focus on the plant-feeding side of the omnivorous feeding strategy. Although we will likely not be able to answer the question, we feel it is appropriate to ask if there are general rules that would guide the design of plant communities to manipulate omnivorous predators in biological control.

Coll, M. and Guershon, M. 2002. Omnivory in Terrestrial Arthropods: Mixing Plant and Prey Diets. *Annual Review of Entomology* 47, 267-297.

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MOVEMENT AND RETENTION OF OMNIVORES MEDIATED BY HABITAT COMPOSITION

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ABSTRACT.

Omnivores provide an interesting twist on the arrestment and patch exploitation theme because they should use resources at multiple trophic levels to inform marginal value estimates. Because foraging modes associated with these resources differ, it is not clear how a patch is defined for these animals. A plant could impact perception of patch quality indirectly, by influencing prey quality or accessibility, or directly, via the nutrition that it provides. Based on habitat composition and quality with respect to adult reproduction and development of offspring, and with respect to previously-measured patch residence times with and without prey, we developed theoretical predictions for retention of omnivores in habitats that differ in both diversity and composition. We tested these predictions for habitats without prey. Based on qualities of the plants, the best predicted habitat composition for reproduction was not the best predicted habitat composition in terms of individual patch residence times. In our habitats, omnivores were retained longer in both types of habitats, and differed between habitats in time spent on different plant types such that it was not possible to reject either hypothesis. Our results demonstrate that predicting the value of the individual habitat components is not straightforward due to the inherent complexity of how omnivores assess habitat value.

INTRODUCTION.

Omnivorous arthropods have complex roles in community dynamics (Polis *et al.* 1989), feeding not just on plants, but also on herbivores and other predators; and are fed upon themselves. Although some theories of trophic control envision distinct trophic levels, omnivores blur these distinctions (Snyder & Evans 2006). Omnivores link multiple trophic levels. The links can be strong and dictate community structure (Paine 1966). In other cases, omnivores have numerous weak but pervasive interconnections with other community members (Polis 1991). In this discussion we restrict our definition of omnivores to animals that feed on both plants and animals (Coll & Guershon 2002), as opposed to the more inclusive definition of omnivores as animals that feed on more than one trophic level (Pimm & Lawton 1974).

Physical constraints dictate that omnivores must alternate feeding effort between resources. In the case of two resources, diet-mixing strategies of an omnivore will depend on whether both resources must be present in its diet for optimum fitness, or whether these resources may substitute completely or partially for

one-another. If two resources are perfectly equivalent, the animal should feed on whichever resource is encountered (Fryxell & Lundberg 1994). If two resources are not perfectly equivalent, then the animal should employ some form of adaptive foraging rule that will allow one resource to substitute for the other (Krivan & Diehl 2005; Diehl 2003), and should budget time and food intake to achieve an optimum ratio of the two resources.

The fitness accrued by an omnivore from residency on a particular plant in the presence or absence of prey can be thought of in terms of fitness gain rate. Fitness gain is (potential fecundity * number of females produced)/development time and fitness gain rate is fitness gain/residence time; or, the accrual of fitness per unit time of residence on the plant. Females should abandon patches (plants) when fitness gain rate drops below the average gain rate for patches in the present environment as expressed in Charnov's marginal value theorem (Charnov 1976). When patch profitability declines below the cost of foraging in the patch, then an omnivore should leave a patch (i.e. patch residence time or giving up time). For omnivores, the patch residence time should depend on an interaction between plant and prey resources such that as resource contributions to omnivore fitness increase, patch residence time should increase (Fig. 1).

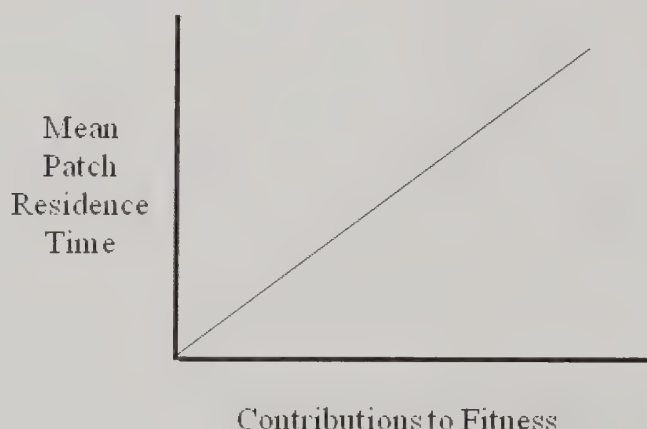


Fig. 1. Hypothetical relationship between patch residence time and resource contributions to fitness in an omnivore.

In nature, omnivores may rarely encounter monocultures of plants. Increasing plant diversity (species richness) generally has positive effects on the abundance of natural enemies (Root 1973, Landis et al. 2000, Fitzgerald & Solomon 2004) but the mechanisms underlying this trend are poorly understood (Sunderland & Samu 2000, Langelotto and Denno 2004). Are effects of diversity due to components of diversity, i.e. one or a few species with strong and direct effects on ecosystems (Houston 1997, Downing and Leibold 2002), or if the effects are due to diversity *per se*, involving more complex interactions between species (Naeem 2002). Compositional effects can be as large, or larger, than the effects of species richness *per se* (Downing & Leibold 2002). If the diversity of plant resources matters to the fitness of omnivores, do omnivores distinguish between diversity *per se* or is it the individual components of the diversity that matter? As omnivores might be managed by such plant diversity, it is important to know how omnivores evaluate plant diversity at a habitat and landscape scale. Our objective in this contribution is to develop and

test theory that explains how small plant feeding omnivores that live on plants might respond to plant diversity at a local (habitat) scales.

Although cumulative fitness gain for these omnivores has not been directly determined, there are at least two ways to rank an omnivore's perception of fitness gain based on plant resources. The first is based on the direct contribution of plant nutritional resources to reproduction and development of the omnivore, and the second is based on the omnivore's estimates of marginal value as determined by patch abandonment times on different plants. In our hypothetical environments, in the absence of prey resources, plants differ in their direct contribution to reproduction of the omnivore, however, with prey resources, the direct value of plant resources to reproduction is minimal and all plants are equal. Additionally, patch residence of omnivores differs between plants and the addition of prey resources has an additive effect on patch residence, but there is no interaction between the plant or prey resources on patch residence. Under the situations of with or without prey, we compare predicted cumulative fitness gain for omnivores in three types of habitats: 1) a monoculture of medium quality plants; 2) a polyculture composed of the monoculture plant species and a high quality plant species, and; 3) a polyculture composed of the monoculture plant species and a low quality plant species (Fig. 2).

In the absence of prey resources, regardless of whether cumulative fitness is based on direct nutritional contribution to reproduction or patch retention times, omnivores should have higher fitness in polyculture habitats with high quality plants, followed by polyculture habitats with low quality plants, and lowest in monoculture habitats if diversity itself is important (Fig. 2). However, if it is the components of diversity that are important, then in the absence of prey, omnivores should have higher fitness in polyculture habitats with high quality plants, followed by monoculture habitats, and lowest in polyculture habitats with low quality plants.

When prey resources are present, omnivores should demonstrate the same pattern as above if cumulative fitness is based on patch retention times, however, fitness would be higher overall due to the additive effect of prey resources (Fig. 2). If fitness is based on direct nutritional contribution to reproduction, then if diversity is important, then fitness should be higher on polycultures than on monocultures. In contrast, if the components of diversity are important, then fitness should be equal regardless of the habitat type, as all plants are equal in the presence of prey resources.

For small plant feeding omnivores that live on plants, the plant is an essentially unlimited resource which cannot be depleted by feeding; although prey on plants can be depleted. One of the touted characteristics of plant-feeding omnivores is their greater tendency to remain in habitats in the absence of prey resources (Coll & Guershon 2002), compared to predators that do not feed on plants. Thus, it is the plant community in the absence of prey that may ultimately determine the tendency of omnivores to remain in habitats. We tested our predictions in the absence of prey using *Dicyphus hesperus* (Knight) (Heteroptera: Miridae) as our model omnivore system. *Dicyphus hesperus* is an omnivore that feeds on a wide variety of plants and soft-bodied insects (Sanchez *et al.* 2004; McGregor *et al.* 1999). This omnivore discriminates between plant species, and in the absence of prey, females prefer to

rest and oviposit on plants that support nymphal development (Sanchez *et al.* 2004). Mullein, *Verbascum thapsus* L. (Scrophulariaceae) supports reproduction, adult

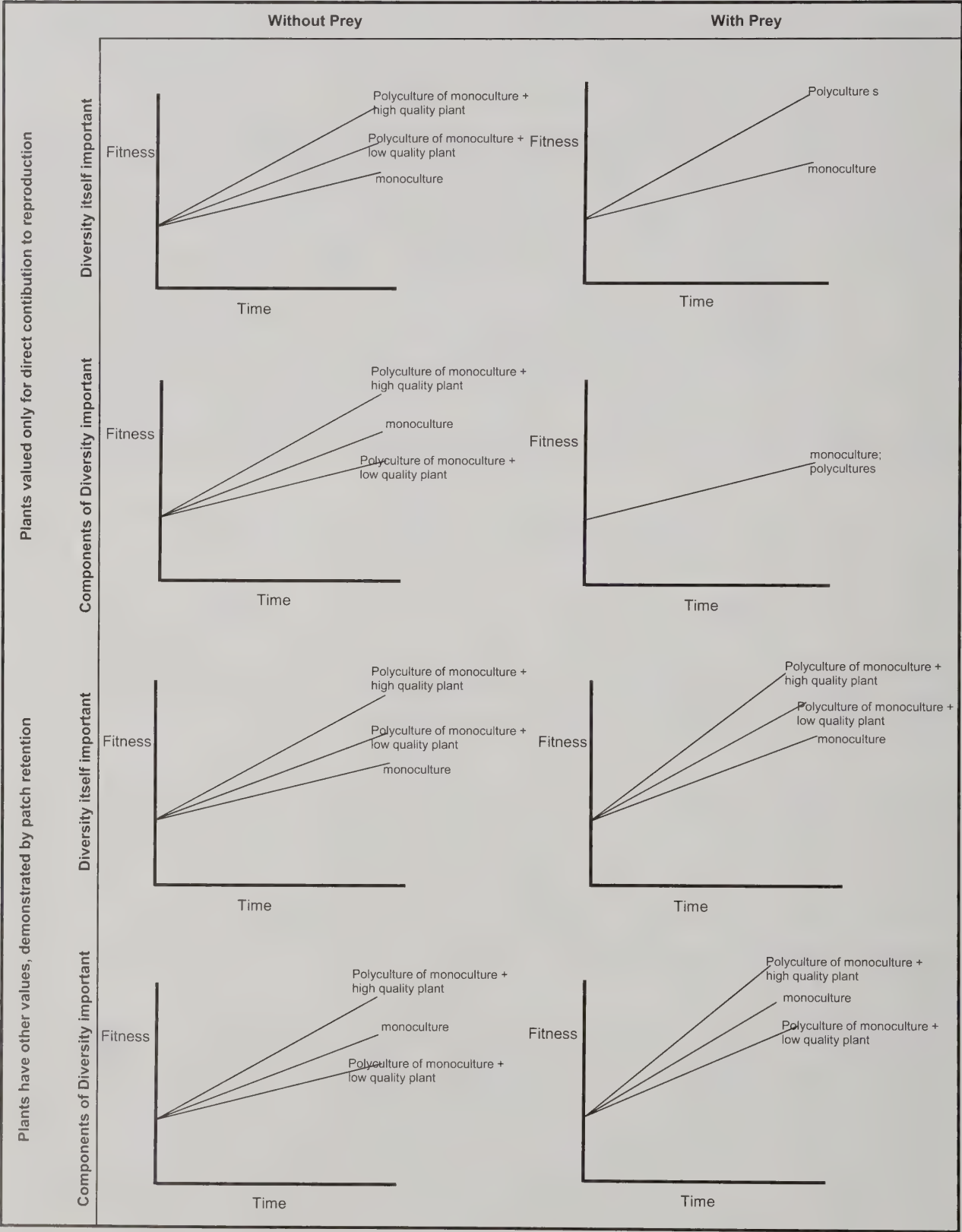


Fig. 2. Hypothesized cumulative fitness gain of omnivores in three types of habitat, when plant contributions to fitness are measured by either their direct nutritional contribution (reproduction) or by their marginal value as assessed by omnivore patch retention.

longevity and nymphal development in the absence of prey. Pepper, *Capsicum annuum* L. (Solanaceae), supports nymphal development to a limited extent in the absence of prey. Tomato, *Lycopersicon esculentum* Miller (Solanaceae), and chrysanthemum, *Chrysanthemum coronaria* L (Asteraceae), do not support nymphal development in the absence of prey. In choice cages without prey, females aggregate on mullein and abandon other plant species (Sanchez *et al.* 2004). However, with unlimited prey, all four plant species equally support reproduction and nymphal development (Sanchez *et al.* 2004). Individual patch-leaving decisions in *D. hesperus* are determined by plant and prey resources independently (VanLaerhoven *et al.* 2006), with leaving times shortest on pepper, followed by chrysanthemum and tomato, and longest on mullein; and plants with prey retaining *D. hesperus* adults longer than plants without prey.

We used habitat retention as a proxy for the value of the habitat for *D. hesperus*, and designed habitats of varying quality. In all cases, mullein was a high quality plant. Based on values for reproduction, pepper was a medium quality plant, followed by tomato then chrysanthemum as low quality plants. Based on patch retention, tomato was a high quality plant, chrysanthemum as a medium quality and pepper as low quality. Based on our predictions in the absence of prey (Fig. 2), we generated a predicted ranking of habitat retention (Fig. 3) using tomato or pepper as the baseline monocultures, which we then tested experimentally.

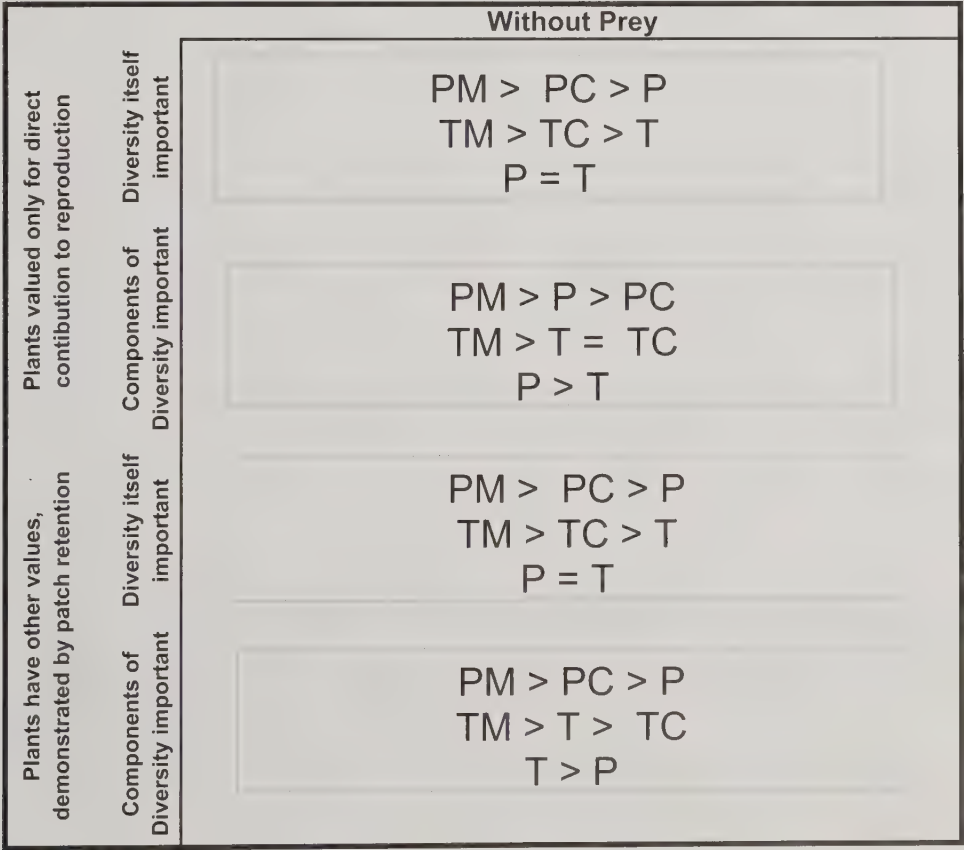


Fig. 3. Predicted habitat retention ranking of *Dicyphus hesperus* based on hypothetical relationship between fitness gain and habitat value in the absence of prey (M=mullein; T=tomato; P=pepper; C=chrysanthemum).

MATERIALS AND METHODS.

The effects of habitat composition on retention were evaluated, in the absence of prey, in habitats containing 17 plants. Plants within habitats were arranged in four rows of four plants each, with one additional plant placed in the geometric center. Each habitat was composed of polyculture treatments (one primary and one secondary plant species) or monoculture treatments (all one primary plant species). In polycultures, the primary plant species were either tomato or pepper and represented 13 of the 17 plants within the arena. Secondary plant species were either mullein or chrysanthemum. When present, secondary plants comprised four of the 17 plants in the arena, with one placed randomly per row.

Three mated 72 h starved females (4 day old) were marked with a combination of two colors using red, blue, yellow and white, luminous dusting powder (BioQuip, Rancho Dominguez CA) to allow for individual tracking of movement and then released on the centre plant (either pepper or tomato) within an arena. Each omnivore was checked each morning to determine its host-plant location within the habitat until it left the habitat. Once all three omnivores had left the habitat, a new group of 3 omnivores was released on the centre plant. Omnivores found outside the habitat (i.e. 30 cm or more from the nearest plant) were removed so they could not re-enter the habitat. Any omnivore found with the incorrect color pattern for that particular habitat was removed. No arthropod prey were introduced to the plants and plants were checked daily for presence of prey and these were removed when found. A total of 36 omnivores were tested for each plant combination.

Based on preliminary experiments, each omnivore was considered to be an independent test of the hypothesis. Effects likelihood ratio chi-square test within Cox proportional hazard regression analysis was used to test the effects of primary and secondary plant type on the probability of leaving the habitat. Log-ranked chi-square survivor analysis was used to separate effects determined to be significant using Cox proportional hazard analysis i.e. analogous to using Tukey HSD means separation after ANOVA. For all tests, $\alpha = 0.05$. All statistical analyses were conducted using JMP 7.0 (SAS 2007).

RESULTS.

Mean habitat retention time was not affected by primary plant species, however, there was an effect of secondary plant species and an interaction effect (Table 1). In tomato habitats, retention time was longer in habitats with mullein than those with chrysanthemum, however monoculture tomato habitats were not different from habitats with tomato & mullein or tomato & chrysanthemum. In pepper habitats, retention time was longer in habitats with mullein, than those with chrysanthemum or no secondary plant species. Retention time of omnivores was longer in pepper and mullein habitats, than in tomato and mullein habitats ($\chi^2_1=7.873$, $P=0.005$).

Table 1. Mean (\pm SE) habitat retention time by omnivores in tomato, tomato-chrysanthemum, tomato-mullein, pepper, pepper-chrysanthemum and pepper-mullein habitats.

Treatment	Mean (±SE)	Significant Effects
Primary Plant		
Tomato	8.4 ± 0.4	χ² ₁ =0.069, P=0.793
Pepper	10.0 ± 0.7	
Secondary Plant		
Tomato & Mullein	10.6 ± 1.1 a	χ² ₂ =38.711, P<0.0001
Tomato & Chrysanthemum	6.9 ± 0.6 b	
Tomato alone	7.8 ± 0.7 ab	
Pepper & Mullein	16.7 ± 1.9 a	χ² ₂ =12.073, P=0.002
Pepper & Chrysanthemum	6.6 ± 0.6 b	
Pepper alone	5.9 ± 0.4 c	
interaction P*S		

DISCUSSION.

For omnivores such as *D. hesperus*, plant composition within a habitat clearly affects the omnivore’s estimate of fitness in that habitat, based on residence time, and the effort given to exploring the habitat. Using pepper as the monoculture, we were unable to distinguish between three of our four hypotheses (Fig. 4). Using tomato as the monoculture provided support for our components of diversity/aggregate patch retention hypothesis. However, we are not able to exclude the other hypotheses. Further research incorporating prey into the habitats may provide the insight required to better understand how omnivores assess habitat composition and complexity.

CONCLUSIONS.

The accumulation of individual omnivores in habitats is likely to be a result of retention due to direct compositional effects of individual plants as nutrients and hosts for reproduction and development, as well as other effects such as potential for prey resources, refugia from predation, abiotic conditions and locations to find mates. Because the plant community provides the first information about the quality of the habitat for an omnivore, the willingness of an omnivore to explore the habitat should be determined initially by plant species composition and subsequently by prey discovery. As we have demonstrated here, interactions of omnivores with other members of the food web can be complex, even at these scales, due to multiple direct and indirect interactions inherent to omnivores.

Predicted			Actual
Plants valued only for direct contribution to reproduction	Diversity itself important	PM > PC > P TM > TC > T P = T	PM > PC > P TM = T > T = TC P = T
	Components of Diversity important	PM > P > PC TM > T = TC P > T	
Plants have other values, demonstrated by patch retention	Diversity itself important	PM > PC > P TM > TC > T P = T	
	Components of Diversity important	PM > PC > P TM > T > TC T > P	

Fig. 4. Comparison of predicted and actual habitat retention rankings of *Dicyphus hesperus*.

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PLANTS AS HETEROGENEOUS RESOURCES FOR OMNIVOROUS BUGS IN CONSERVATION BIOLOGICAL CONTROL

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ABSTRACT.

To enhance the activity of naturally-occurring natural enemies in agroecosystems, we need to understand the way they respond to variability in resources in their habitat. True omnivores, that feed on both prey and plant resources, present applied researchers with particular challenge, stemming from their response to variation in plant quality at the within- as well as between-plant scales. Here we explore the ability of two omnivorous anthocorid bugs (Heteroptera) to assess variations in the quality of plant resources they use as food or oviposition media. We then discuss how these insights could be used in conservation biocontrol programs.

In our first study, in the laboratory, *Orius albidipennis* females preferentially oviposited in a particular part of cotton leaves, which they defended against conspecific intruders. Further, they defended these sites more successfully on nitrogen-rich than nitrogen-poor plants. In our second study, *Anthocoris nemoralis* exhibited oviposition preference for some Mediterranean tree species over others, which corresponded with egg hatching success. Contribution of pollen to omnivore survival and fecundity differed among tree species.

The spatio-temporal response of these omnivores to availability of plant resources is being harnessed now to enhance their activity in conservation biocontrol programs.

INTRODUCTION.

Many predators feed on plant materials to supplement their prey diet. Some of these true omnivores (i.e., consumers that feed on both prey and plants) also use plants as oviposition substrate. Plants provide omnivores with highly heterogeneous resources. At the within-plant scale, some plant parts and structures may provide higher quality food resource or better protection for eggs, than other parts (Evans 1976). At the population level, individual plants are likely to differ in their nutritional value, which is particularly important for omnivorous species in which early instars feed exclusively on plant material (Parker 1981). Finally, at the plant community scale, a variety of plant resources of different qualities become available at different times. Thus omnivores may be exposed to different plant resources at different parts of their life cycle. Variation in plant resource availability and quality in time and space is therefore expected to greatly impact omnivores' performance and population dynamics. This is especially important for conservation biocontrol, in which the

environment is manipulated to enhance the establishment and population build-up of omnivorous biocontrol agents in crop habitats.

Many omnivorous anthocorid bugs (Heteroptera: Anthocoridae) are important predators of key agricultural pests and are commonly used as biocontrol agents in conservation and augmentation biocontrol programs. We use here our recent work on two anthocorid bugs. The first, *Orius albidipennis* Reuter, is a generalist predator which preferentially preys on thrips (especially the pestiferous western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)) (Rubin *et al.* 1996). The second is *Anthocoris nemoralis* (F.), which preferentially feeds on psyllids (Hodgson & Aveling 1988), including the pear psylla, *Cacopsylla bidens* (Sulc) (Hemiptera: Psyllidae), a major pest in commercial pear orchards (Brunner & Burts 1975). Both anthocorids benefit from supplementing their prey diets with pollen or other high quality plant parts such as bean pods (Vacante *et al.*, 1997, Shaltiel & Coll, in prep.). We tested the ability of the two anthocorids to assess variation in plant resource quality by monitoring their oviposition on various structures within as well as between plants; *O. albidipennis* was tested for its ability to discriminate between cotton foliage of low- and high-nitrogen content, and *A. nemoralis* was offered choice between four Mediterranean tree species. We also studied the suitability of plant resources serving as foods for *A. nemoralis* adults and *O. albidipennis* nymphs. Finally, we tested whether oviposition site selection by the two omnivores corresponds with offspring performance, and discuss how results may be applied to enhance conservation biocontrol of their pestiferous prey.

METHODS.

Insects and plants.

Orius albidipennis culture was established with adults collected from sunflower heads in the Arava Valley in Southern Israel, and maintained at $25 \pm 1^\circ\text{C}$, $70 \pm 10\%$ RH and 16L:8D h following published protocols (Schmidt *et al.* 1995). While in the culture, *O. albidipennis* were fed *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, and provided with bean pods as dietary supplement and oviposition substrate.

Cotton plants *Gossypium hirsutum* L. (Malvaceae) were grown in the greenhouse from seeds, in inert rooting mixture (Vermiculite 2[®]). At the three-node stage, the plants were randomly assigned to either rich or poor nitrogen fertilization regime, which resulted in nitrogen-rich and nitrogen-poor plants, with significantly different leaf nitrogen contents (for details see Groenteman *et al.*, 2006).

Anthocoris nemoralis culture was established from adults collected on various trees in the research area in the upper Galilee, held in groups of ten pairs in ventilated plastic containers (15x20 cm) maintained at $22 \pm 3^\circ\text{C}$, 16:8 L: D and 70% RH. Laboratory experiments were held under these conditions. The culture was transfused occasionally with field-collected material. The bugs were fed eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (frozen and U.V. sterilized) and honey, and provided with moist cotton wicks. Lima bean pods were provided as oviposition substrate, and were collected daily to synchronize nymph age.

Pollen was collected during the respective flowering period, from four tree species: laurel, *Laurus nobilis* L. (Lauraceae), evergreen buckthorn, *Rhamnus alaternus* L. (Rhamnaceae), mastic tree, *Pistacia lentiscus* L. (Anacardiaceae), and wild pear, *Pyrus syrica* Boiss. (Rosaceae), and kept at -20°C until used in the experiments.

Orius albidipennis.

Defensive behavior at oviposition site. Interactions between *O. albidipennis* females were studied at a site on the leaf identified as preferred, a 0.5 cm diameter area at the vein origin (Groenteman *et al.* 2006). Twenty, mated females (6 ± 1 days old) were randomly collected from the culture and placed individually in Petri dishes containing an intact cotton leaf, either nitrogen-rich or nitrogen-poor, and allowed to settle for 20 min. By that time, most females had settled in the vein origin area and were therefore labeled as “owners”. Another female, labeled as “intruder”, was then introduced into each dish, and the interaction between the two females was observed. The proportion of victories achieved by “owners” and “intruders” on nitrogen-rich and nitrogen-poor leaves were χ^2 tested. To test the importance of size to contest outcome, we χ^2 tested the difference between interacting females’ head width, prothorax width and fore tibia length.

Oviposition site selection and egg hatching success. Twenty, mated *O. albidipennis* females (6 ± 1 days old) were randomly collected from the culture, placed individually in Petri dishes, each containing an intact nitrogen-rich or nitrogen-poor cotton leaf, and allowed to oviposit for six days. Leaves were replaced on the third day to prevent cannibalism on newly hatched nymphs. Egg deposition per female per day was recorded, as well as egg location on the leaf, either at the vein origin zone or at the rest of the leaf lamina. The number of eggs deposited per cm² of leaf was compared for the two nitrogen treatments and analyzed using ANOVA with a split-plot model (nitrogen treatment as whole plots and leaf-zone as subplots). Egg hatching success was determined after 5 additional days. The proportion of hatched eggs (arcsine of square root transformed) was subjected to ANOVA with the same split-plot model.

Nymph performance. Newly hatched *O. albidipennis* nymphs were individually enclosed in plastic tubes (12 mm diameter \times 7 mm height) on the underside of intact nitrogen-rich or nitrogen-poor cotton leaves. Four such enclosures were used per leaf and three leaves were used per nitrogen treatment. Nymphs were inspected every 4-10 h over the course of two days, and their survival and development were monitored. Observation ceased when a nymph either died or had reached the second instar. Percent mortality on nitrogen-rich and nitrogen-poor leaves was analyzed using ANOVA with nymphs nested within leaves. Duration (in hours) of the first stadium on the two nitrogen treatments was compared using Student t-test.

Anthocoris nemoralis

Population dynamics and resource availability in Mediterranean woodland. Arthropod community was monitored on the four experimental tree species using the beating tray method (Burts & Retan 1973). Ten trees of each species were randomly selected along a 30 km transect in the upper Galilee. The same trees were samples

every other week, year-round from 1 February 1998 until 31 December 2000. In each sample, three branches of each tree were struck three times with a stiff plastic hose covered with polystyrene foam, over an off-white plastic tray (0.3 x 0.4 m). The dislodged insects collected on the tray under each branch constituted a sample, and were counted and identified. Developmental stage of *A. nemoralis* specimens was recorded as either nymphs or adults. All insects were then released back onto the sampled tree. *A. nemoralis* population density (corrected for average volume and relative frequency of each tree species, and square-root transformed) on the four tree species was compared using one-way ANOVA.

Oviposition preference and egg hatching success. The oviposition preference of *A. nemoralis* females for different tree species was tested in a choice experiment. On each replication, 3-5 mated females (4 days old) were released into a cage containing 10 cm long branches (leaf area was standardized to 90 cm²) from two of the test trees. During the experiment, the females were provided with unlimited prey (*E. kuehniella* eggs) on cage floor. The number of eggs deposited on each branch was determined under a stereomicroscope four days later. The experiment was replicated five times for each of the six plant-pair combinations. Each female was used only once in the experiment. The experiment was conducted in the spring, when all tree species had fresh foliage. The effects of the combination of tree species on the number of eggs per female (square root transformed) were tested in two-way ANOVA and means were ranked using Tukey-Kramer HSD test ($\alpha = 0.05$).

At the end of the oviposition choice experiment, the females were removed from the cages and each branch (n=60) was held separately for eight more days. The number of newly emerged nymphs was determined daily. The effect of tree species on percent hatch (arcsine of square root transformed) was tested using one-way ANOVA.

Female survival and fecundity. To test the effect of different plants and prey diet combinations, on female survival and fecundity, 4th instar nymphs were kept individually in ventilated plastic containers and provided with one of the following 16 food combinations (four diet treatments in each of the four tree systems): foliage only; foliage + pollen; foliage + pollen + psylla; psylla only. In addition to the unique pollen of each tree, we used the corresponding specialist psylla spp. found on each tree species: *Cacopsylla bidens* on pear, *C. myrthi* (Puton) on buckthorn, *Trioza alacris* Flor on laurel, and *Agonoscena* spp. on mastic tree. Moist cotton wicks were provided in all treatments. The survival and development of the bugs were recorded daily until adulthood. Upon reaching adulthood, each caged female was joined by two males for 24 hours, to insure insemination. Six days later, the females were dissected and the number of oocytes and mature eggs were recorded. The experiment was replicated 14 times in a complete randomized block design with a total of 224 female nymphs. The effect of diet on the number of eggs and oocytes (square root transformed where necessary) was analysed using one-way ANOVA. Means were ranked using Tukey-Kramer HSD test ($\alpha = 0.05$).

RESULTS.

Orius albidipennis

Defensive behavior at oviposition site. Mated *O. albidipennis* females showed a tendency to settle at the vein origin site on both nitrogen-rich and nitrogen-poor leaves. “Owner” females on nitrogen-rich leaves prevailed significantly more often in intraspecific conflicts than “intruding” females ($\chi^2_{19} = 6.25$, $P = 0.01$). On nitrogen-poor leaves, however, “owner” and “intruding” females had a similar chance of winning ($\chi^2_{19} = 2.57$, $P = 0.1$). Female size did not correlate with the chance to win a conflict on neither nitrogen-rich nor nitrogen-poor leaves ($\chi^2_1 = 2.2$, $P = 0.14$ and $\chi^2_1 = 0.03$, $P = 0.88$ for difference in prothoracic size on nitrogen-rich and nitrogen-poor leaves respectively; differences in head and fore tibia sizes behaved similarly).

Oviposition site selection and hatching success. *Orius albidipennis* females deposited significantly more eggs per leaf area at the vein origin region than on the rest of the leaf ($F_{1,18} = 45.19$, $P < 0.001$, Table 1). Nitrogen treatment did not affect overall egg deposition rate (1.68 ± 0.27 and 1.46 ± 0.27 on nitrogen-rich and nitrogen-poor leaves respectively; $F_{1,18} = 0.501$, $P = 0.488$), and there were no significant interacting effects of nitrogen treatment and leaf region on oviposition rate ($F_{1,18} = 0.33$, $P = 0.575$).

Significantly more eggs hatched at the vein origin region than on the rest of the leaf, both on nitrogen-rich and nitrogen-poor leaves (Table 1; $F_{1,36} = 15.83$, $P < 0.001$). Nitrogen fertilization regime did not affect overall egg hatching success ($F_{1,32} = 1.88$, $P = 0.18$) and there was no significant interacting effect of nitrogen treatment and leaf region on egg hatch ($F_{1,36} = 0.159$, $P = 0.69$).

Table 1. Density (means \pm 1 SE) of *Orius albidipennis* eggs and percent of eggs hatched (means \pm 1 SE) in two regions of cotton leaves grown under low or high nitrogen fertilization regimes.

Site on leaf	Area (cm ²)	Eggs (no. /cm ²) ^a		Percent hatched eggs ^a	
		Nitrogen rich	Nitrogen poor	Nitrogen rich	Nitrogen poor
Vein origin ^b	0.196	4.58 \pm 0.99a	5.12 \pm 0.96a	59.9 \pm 8.6a	60.8 \pm 8.7a
Leaf lamina ^c	12.37	0.04 \pm 0.01b	0.03 \pm 0.01b	29.7 \pm 8.6b	30.2 \pm 8.7b

^a Values within columns followed by the same letter do not differ significantly (F tests, $P < 0.001$). Within rows and variable (egg deposition and egg hatch) values for high- and low-nitrogen plants did not differ significantly.

^b An area of 0.5 cm diameter tangent to the petiole.

^c An area of 4 cm diameter tangent to the petiole, excluding the vein origin.

Nymph performance. Nymph survival on nitrogen-rich leaves was about 31% longer on average than on nitrogen-poor leaves. This difference however was not statistically significant (41.4 ± 4.7 and 31.5 ± 4.4 h, respectively; $F_{1,20} = 2.57$, $P = 0.137$). Duration of the first stadium was significantly shorter on nitrogen-rich than on nitrogen-poor leaves (20.7 ± 2.7 and 38.9 ± 4.3 h, respectively; $t_5 = -3.59$, $P = 0.016$).

Anthocoris nemoralis

Population dynamics and resource availability in Mediterranean woodland: A. nemoralis density varied greatly both seasonally and spatially between tree species (Fig. 1A). Adult population persisted throughout the year on *P. lentiscus* and peaked in late spring on all tree species. Adult population was lowest on *P. syrica*.

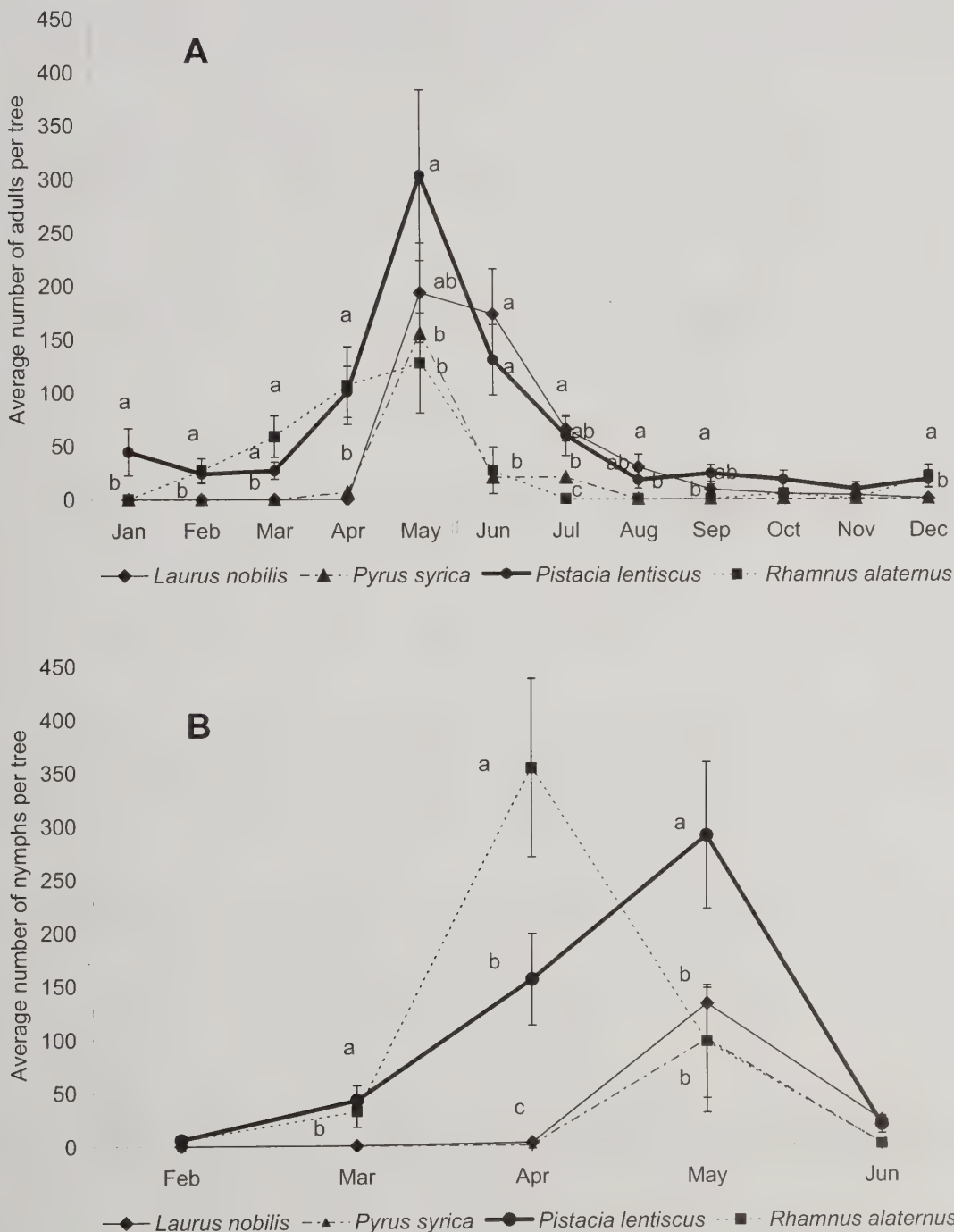


Fig. 1. Mean density (± SE) of *Anthocoris nemoralis* (A) adults and (B) nymphs on four Mediterranean tree species. Data were collected twice per month for 3 years and corrected for tree volume and relative frequency in the wood. Marks followed by same letter within a month do not differ significantly (p<0.05).

Nymphs started to appear in low numbers in February and could be found throughout summer (Fig. 1B). Nymph population increased most rapidly on *R. alaternus*. They peaked on this tree in April and disappeared in June. Relatively few nymphs were found on *P. syrica* throughout the growing season. Overall, there was a significant difference in *A. nemoralis* density on the four tree species (Table 2).

Oviposition preference and egg hatching success. *A. nemoralis* females preferred to deposit their eggs on *R. alaternus* and *L. nobilis*. *Pyrus syrica* was the least preferred for oviposition (Table 2). Significantly more eggs hatched on *P. lentiscus* and *L. nobilis* than on *P. syrica* (Table 2).

Table 2. Ranked *Anthocoris nemoralis* preference for and performance on four Mediterranean tree species. Ranking is based on statistical differences between trees within variables (rows). Data will be published elsewhere.

Parameter	<i>Pistacia lentiscus</i>	<i>Rhamnus alaternus</i>	<i>Laurus nobilis</i>	<i>Pyrus syrica</i>
Field density	a	b	b	c
Oviposition preference	ab	a	a	b
Hatching success	a	ab	a	b

Female survival and fecundity. The effect of prey and plant diet combination on female survival and fecundity varied among tree species (Tables 3, 4). On *R. alaternus*, the females survived longest when fed pollen and their fecundity was highest and similar on pollen or psylla diets. On *P. lentiscus* and *L. nobilis*, however, plant foods were inferior to prey in their effect on female survival and fecundity (Tables 3, 4).

Table 3. Ranked relative survival of *Anthocoris nemoralis* females on different plant and prey combinations on four Mediterranean tree species. Relative survival is ranked based on statistical differences between diets. Data will be published elsewhere.

Plant species	Foliage	Foliage +pollen	Psylla	Foliage +pollen +psylla
<i>Pistacia lentiscus</i>	b	ab	a	a
<i>Rhamnus alaternus</i>	b	a	b	a
<i>Laurus nobilis</i>		b	a	a
<i>Pyrus syrica</i>	b	ab	a	a

Table 4. Ranked relative fecundity (no. of eggs and oocytes) of *Anthocoris nemoralis* females reared on different plant and prey combinations on four Mediterranean tree species. Relative fecundity is ranked based on statistical differences between diets. Data will be published elsewhere.

Plant species	Foliage	Foliage +pollen	Psylla	Foliage +pollen +psylla
<i>Pistacia lentiscus</i>	b	b	a	ab
<i>Rhamnus alaternus</i>	b	ab	b	a
<i>Laurus nobilis</i>		b	a	a
<i>Pyrus syrica</i>	b	ab	ab	a

DISCUSSION.

Our studies show that omnivorous anthocorids respond to variation in the quality of plant resource at different scales. At the within-plant scale, *O. albidipennis* females preferentially deposited eggs at the vein origin region of cotton leaves, possibly due to the more succulent nature of this structure. Eggs embedded at this oviposition site may be better protected from desiccation. This structure is also likely to protect the eggs from predation, as previously shown in another omnivorous anthocorid, *Anthocoris confusus* (Reuter) (Evans 1976). Likewise, our results indicate that eggs at the vein origin region are more likely to hatch successfully than eggs deposited in other parts of the leaf. Also at the within-plant scale, variation in food source quality affected *A. nemoralis* performance. On all tested plants, foliage was inferior food for the omnivore compared to pollen. Furthermore, *Rhamnus alaternus* pollen was a superior food source for the bug even when compared to the psylla prey available on that tree; the females survived and reproduce better on pollen than on psylla on this tree. At the between plant scale, *O. albidipennis* females were able to distinguish between nitrogen-rich and nitrogen-poor cotton plants. This was evident from their greater success in defending preferred oviposition sites on high- vs. low-nitrogen leaves. At the plant community scale, different densities of *A. nemoralis* were recorded on different tree species in the woods. This is possibly the result of the combination of three factors: (a) preferential oviposition on some tree species but not others; (b) variation in egg hatching success and in bug survival and fecundity on different tree species; and (c) variation in food source availability and quality on these tree species.

For both omnivore species, oviposition preference appears to be correlated with performance. *Anthocoris nemoralis* for example, least preferred to oviposit on wild pear, where egg hatch and female survival and fecundity were inferior compared to the other tree species. *Orius albidipennis* nymphs performed better on nitrogen-rich cotton leaves than on nitrogen-poor ones, which corresponds with female defensive behavior.

Appreciating the importance of temporal and spatial heterogeneity in plant resources to anthocorids and other omnivorous predators is the first step toward harnessing these natural enemies to suppress pests in conservation biocontrol programs. Work by Shouster (2003), for example, showed that weedy stands of golden crownbeard, *Verbesina encelioides* (Cav.) A. Gray (Asteraceae) near strawberry fields, supports *Orius niger* populations. The bug migrates to adjacent strawberry fields and successfully reduces *Frankliniella occidentalis* densities to below damaging levels. Results show that *V. encelioides* flowers before strawberry, thus supporting early population build up of *Orius* bugs. The bugs feed on high quality *V. encelioides* pollen, as well as on non-pestiferous thrips prey. Migration of adult *Orius* to adjacent strawberry fields could be encouraged by mechanical felling of *V. encelioides* stands.

In a perennial system, *Anthocoris nemoralis* success to reduce populations of the pear psylla, *C. bidens*, varies in different parts of the world, and depends primarily on early-season migration of the bug in sufficient numbers into the orchard (Solomon *et al.* 2000). Results presented here and other data (Shaltiel 2002) show

that *R. alaternus* is a preferred host for *A. nemoralis* oviposition, that egg hatching success is high on this host, and that the tree supports high *A. nemoralis* populations early in the season. Shaltiel & Coll (2004) therefore recommend planting *R. alaternus* hedges around pear orchards to serve as source habitat for the omnivore; *Anthocoris nemoralis* abandon *R. alaternus* trees soon after they stop flowering, and is expected then to colonize the orchard. This practice has several advantages: on *R. alaternus*, *A. nemoralis* preys on *Cocopsylla merthi* Puton, which is highly specific and does not infest pear trees (Halperin 1988). In addition, *C. merthi* is univoltine, and its populations collapse before pear psylla reach peak density (L. Shaltiel, pers. obs.). Finally, *R. alaternus* hedges could consist of male plants only, which would provide *A. nemoralis* with high-quality pollen resource, while avoiding the risk of seed production and seedling invasion into the orchard (Shaltiel *et al.* 2004). This practice is currently being trialed in commercial pear orchards near Rosh-Pina, in northern Israel.

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POPULATION DYNAMICS CONSTRAINTS IN AN OMNIVORE DICYPHINE

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ABSTRACT.

The population dynamics of a *Dicyphus* sp. were quantified in tomato settings in the presence and absence of pumpkin as a companion plant. The assay was carried out in two greenhouses with five compartments each. Three of the compartments had only tomato (*Solanum esculentum*) plants, and the other two had tomato and pumpkin (*Lagenaria siceraria*). *Dicyphus* was released in one of the compartments with only tomato plants and in all of those with pumpkin. In one of these compartments, pumpkin was cut once the *Dicyphus* population had built up. *Macrolophus pygmaeus* was released in one compartment with tomato only. Each set-up was replicated twice. In each compartment, mirids were counted on 15 whole tomato plants. Six whole pumpkin plants were sampled for *Dicyphus*. Translucent sticky traps were placed on the outside of each compartment. The *Dicyphus* population on tomato was much lower than that of *M. pygmaeus*. On pumpkin, *Dicyphus* reached populational peaks similar to those of *Macrolophus* on tomato. When pumpkin plants were cut, there was a small increase in the number of *Dicyphus* on tomato, but the number of *Dicyphus* moving from tomato to pumpkin was lower than expected. The captures of *Dicyphus* on sticky traps were significantly higher in the compartments with still-intact pumpkin plants than in the rest of the compartments.

INTRODUCTION.

Vegetation diversity has generally been outlined as one of the key factors for the abundance of natural enemies in crops (Altieri & Letourneau 1982; Andow 1991; Wilkinson & Landis 2005). Plant-provided food (i.e. pollen, nectar) and prey have been considered as the main factors driving omnivorous insect population dynamics (i.e. Van Rijn & Sabelis 2005), but little attention has been given to the impact that the preference for host plants may have on their population dynamics and distribution, and on the way they exploit resources within systems. Several species of omnivorous dicyphini (Heteroptera: Miridae) are common in the Mediterranean area, where they naturally colonise crops when the use of wide-action pesticides is reduced (Alomar *et al.* 1994; Tavella *et al.* 1997; Sanchez *et al.* 2003a). The variation in the abundance and timing of colonisation by omnivorous dicyphine of tomato (*Solanum esculentum* (Miller), Solanaceae) crops has been found to be largely related to their population size in the surrounding environment (Castañé *et al.* 2004). However, the abundance of predatory mirids in adjacent field surroundings does not always ensure an earlier and higher crop colonisation, and the factors that influence these movements are still to be investigated (Alomar *et al.* 2002).

Diciphyni have a very restricted number of host plants, including many species with a dense distribution of hairs and/or glandular trichomes, the Solanaceae, Scrophulariaceae, Lamiaceae and Asteraceae being among the best-represented families (Alomar *et al.* 1994; Cassis 1986; Martínez-Cascales *et al.* 2006; Sanchez *et al.* 2006; Schuh & Slater 1995; Wheeler 2001). Most of the North American species of the genus *Dicyphus* have been collected on Solanaceae and Scrophulariaceae (Cassis 1986). In the Mediterranean area, *Macrolophus* and *Dicyphus* species are associated with specific plants throughout their wide geographical distribution, many of them belonging to the Solanaceae, Lamiaceae and Asteraceae (Alomar *et al.* 1994; Tavella *et al.* 1997; Martinez-Cascales *et al.* 2007; Sanchez *et al.* 2003a, 2006). The significance of host preference in Dicyphini is not clear, yet. The sticky nature of many of their hosts might exclude a great number of potential predators and provide a natural, enemy-free space. However, other factors, such as the ability to develop and survive during long periods feeding only on plants, seem to be of great importance in the selection of the host (Sanchez *et al.* 2004). Host preference has been exploited to improve the performance of *Dicyphus hesperus* Knight in tomato crops, by introducing mullein (*Verbascum thapsus* L., Scrophulariaceae) as a companion plant in tomato greenhouses, in British Columbia, Canada (Sanchez *et al.* 2003b).

We showed how host preference in a *Dicyphus* species (Heteroptera: Miridae) from the *Dicyphus hyalinnipennis* group (*sensu* Wagner 1964) influences its population dynamics and movements between crop and companion plants. This *Dicyphus* species is commonly found on pumpkin (*Cucurbita maxima* L. and *Lagenaria siceraria* (Molina) Standley (Cucurbitaceae)), and, at lower numbers, in tomato in traditional polyculture in south-east Spain. In the laboratory, we observed that it was a good predator of the whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) and could be mass-reared without any problem, both on tomato and pumpkin, with *Ephestia kuehniella* Zeller eggs as prey (J. A. Sanchez, unpub. data).

MATERIALS AND METHODS.

The assay was carried out during 2005 at the IMIDA research station in Campo de Cartagena (Murcia, Spain), in two 17x11 m "Parral"-type plastic-houses with meshed walls (14x10 threads per cm). The two greenhouses were divided transversally in five compartments, each using the same kind of mesh. The 72 tomato plants of each compartment were arranged in three rows of 24 plants. Plants were separated by 1 and 0.4 m between and within rows, respectively. A Tomato yellow leaf curl Sardinian virus (TYLCSV) and Tomato Spotted Wilt Virus (TSWV) tolerant variety (Bond, Petoseed, Co. Inc. Saticoy, California) was used. Seedlings were transplanted on 21 February 2005. In two compartments (labelled as DTP and DTPc) of each greenhouse, eight plants of pumpkin (*Lagenaria siceraria* (Molina) Standley) were spaced regularly among the tomato plants. The pumpkin plants were guided on trellises in the same way as the tomatoes. The experiment was set up in a completely-randomised design with 2 replications per treatment. *Bemisia tabaci* was introduced at 2, 2, 2, 4, 2, 4 and 4 adults per tomato plant respectively on 1, 7, 14, 21, 27 and 29 April, and 6 May 2005. *Dicyphus* was introduced in one of the compartments (DT) with only tomato plants and in those with pumpkin (DTP and DTPc). In each of these compartments, 36, 36, 72 and 72 adults ($N_{\text{♀♀}}=N_{\text{♂♂}}$) were released respectively on 4, 12, 20 and 26 May 2005. In compartments with pumpkin,

Dicyphus was released on those plants. In one of the compartments (DTPc) of each greenhouse, pumpkin was cut once the *Dicyphus* population had built up (6 June 2005). *Macrolophus pygmaeus* (Rambur) was introduced in one of the compartments (MT) without pumpkin, in the same numbers and on the same dates as *Dicyphus*. No *M. pygmaeus* or *Dicyphus* were released in one compartment (BT) of each greenhouse, that was used as a control for *B. tabaci*.

Dicyphus and *M. pygmaeus* nymphs and adults were counted visually *in situ* every seven days, on 15 whole tomato plants selected at random in each compartment. One middle leaf from each plant was bagged and taken to the laboratory to be inspected under the stereomicroscope, for the counting of whitefly immatures. In all compartments with pumpkin, six whole plants were sampled at random for *Dicyphus* counting. Two translucent sticky traps were placed on each lateral wall, on the outside of each compartment, two days after the cutting of the pumpkin plants. Mirid captures were counted in the following five weeks.

Dicyphus and *M. pygmaeus* populations on tomato and pumpkin were estimated by multiplying the average number of *Dicyphus* per plant by the total number of plants in a compartment. Populations of *Dicyphus* and *M. pygmaeus* on tomato were compared between compartments with and without pumpkin using ANOVA, with date of sampling as covariate. Captures of *Dicyphus* and *Macrolophus* on sticky traps were analysed by ANOVA with date of sampling as covariate. All statistical analyses were performed using the R computing software (R Core Team 2005).

RESULTS.

Dicyphus established early in tomato but their population increased very slowly compared to *M. pygmaeus* (Fig. 1). The maximum populations of *Dicyphus* on tomato in compartments with (DTP) and without (DT) pumpkin were reached in the ninth week after the first release, being 28.8 ± 3.4 and 24.0 ± 6.8 individuals, respectively (Fig. 1). In compartments where pumpkin was cut (DTPc), *Dicyphus* started to increase at a higher rate after the cutting of pumpkin, to reach a population peak of 69.6 ± 8.5 individuals in the two weeks following the cutting of the plants (Fig. 1). Nevertheless, the migration of *Dicyphus* from pumpkin to tomato was lower than expected and, overall, there were no significant differences between treatments in the size of the *Dicyphus* populations on tomato ($F_{2,56} = 1.18$, $P=0.314$). The population of *M. pygmaeus* in tomato reached a peak of 208.8 ± 52.6 individuals six weeks after the release and its population was significantly higher than that of *Dicyphus* in tomato ($F_{3,75} = 10.2$, $P<0.001$). On pumpkin, the *Dicyphus* population increased to reach a maximum of 215.2 ± 11.9 for DTPc and 212.8 ± 78.1 for DTP, between the sixth and eighth weeks, respectively (Fig. 2). In compartments where pumpkin was cut, the *Dicyphus* population decreased slowly and some individuals were still there almost three weeks later, when the pumpkin plants had almost completely dried out (Fig. 2). The number of *Dicyphus* captured on the sticky traps of the external walls of the compartments was significantly higher for compartments with intact pumpkin plants (DTP) than for any of the other compartments (Fig. 3; $F_{1, 42} = 6.36$, $P<0.001$).

The number of whitefly immatures on tomato increased progressively from the beginning of the experiment until the end (Fig. 4). However, there were no significant differences in the number of whitefly between any of the treatments ($F_{1, 85} = 0.763$, $P=0.552$).

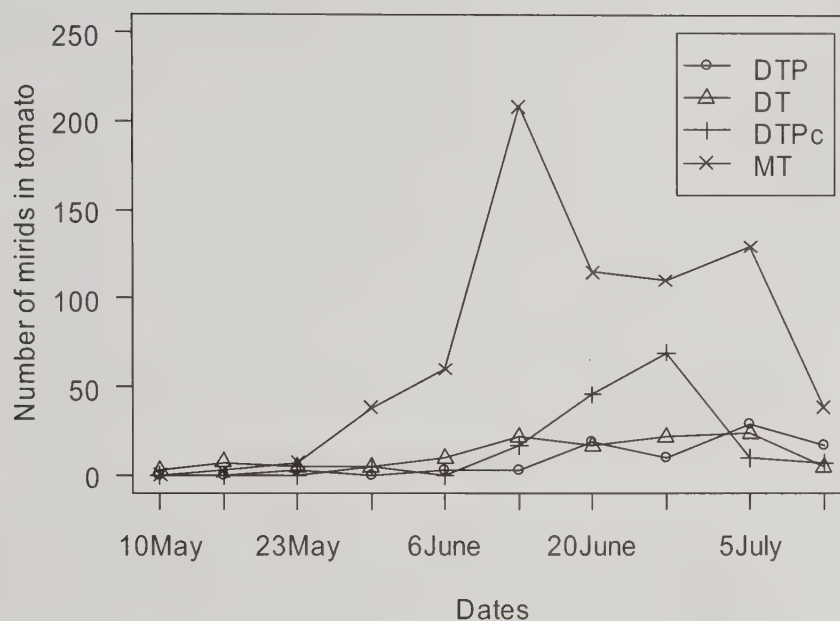


Fig. 1. Population dynamics of *Dicyphus* and *Macrolophus* on tomato. DTP = evolution of *Dicyphus* in compartments with tomato and pumpkin; DT = *Dicyphus* in compartments with tomato; DTPc = *Dicyphus* in compartments with tomato and pumpkin cut on 6 June 6; MT= *M. pygmaeus* in compartments with only tomato.

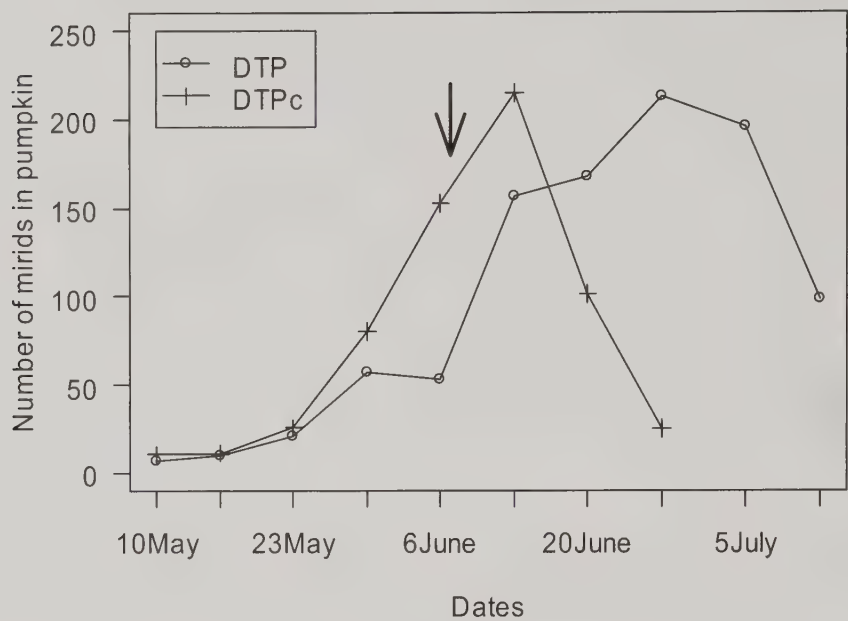


Fig. 2. Population dynamics of *Dicyphus* on pumpkin. DTP = evolution of *Dicyphus* in compartments with tomato and pumpkin; DTPc = evolution of *Dicyphus* in compartments with tomato and pumpkin cut on 6 June (arrow).

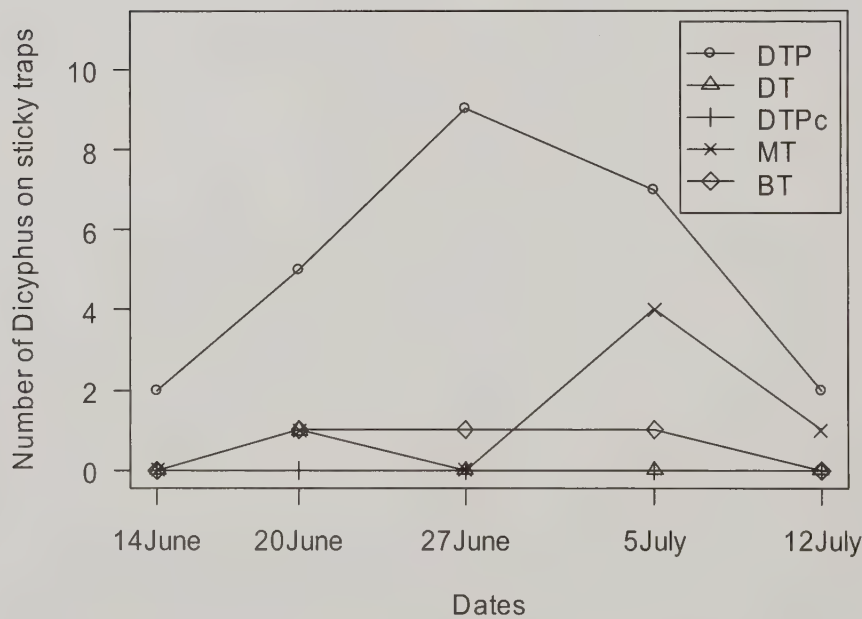


Fig. 3. Captures of *Dicyphus* on sticky traps. DTP = compartments with *Dicyphus*, tomato and pumpkin; DT = compartments with *Dicyphus* and tomato; DTPc = compartments with *Dicyphus*, tomato and pumpkin cut on 6 June; MT= compartments with *M. pygmaeus* and tomato; BT= control.

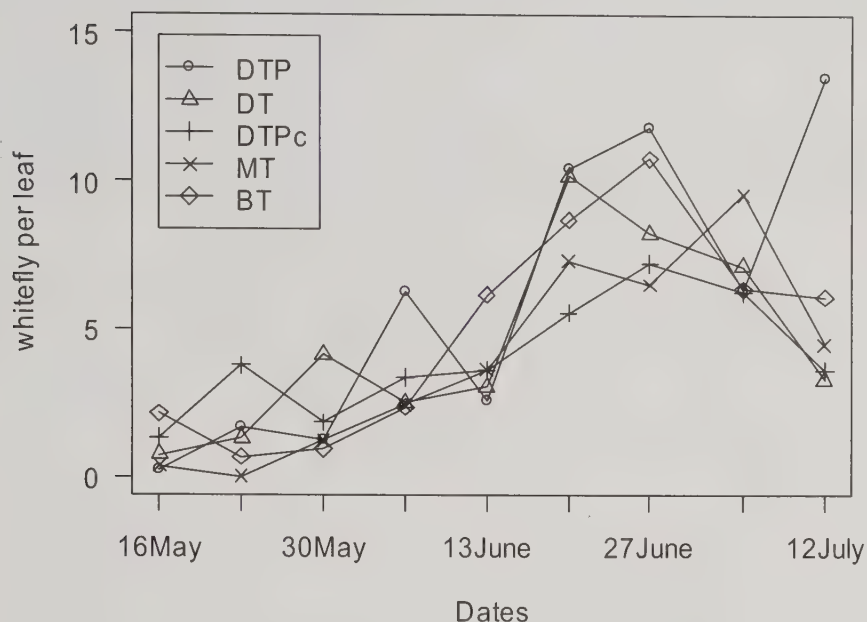


Fig. 4. Evolution of whitefly immatures on tomato; DTP = evolution of whitefly in compartments with *Dicyphus* tomato and pumpkin; DT = with *Dicyphus* and tomato; DTPc = with *Dicyphus*, tomato and pumpkin cut on 6 June; MT= with *M. pygmaeus* and tomato; BT= control.

DISCUSSION.

We observed that the abundance and movements of a *Dicyphus* species belonging to the *Dicyphus hyalinipennis* group (*sensu* Wagner 1964) in tomato crops, with and without pumpkin as companion plant, were greatly influenced by the host plant species. On pumpkin, *Dicyphus* reached a population size similar to that of *M. pygmaeus* on tomato, while on tomato its numbers were very low irrespective of the presence of pumpkin as a companion plant. Many authors have argued that the higher the abundance of natural enemies in the surroundings, the earlier their colonisation of crops would take place and the greater would be their abundance (Altieri 1982; Wilkinson & Landis 2005). However, this study provides data that argues against the generality of this statement, as the presence of a large population of a predatory mirid on the companion plant did not translate into a higher abundance in the tomato crop. The same kind of behaviour has been observed in other mirid species, and in dicyphini in particular. Arnó *et al.* (2000) observed that tobacco plants could be used as insectary plants to maintain populations of *Macrolophus melanotoma* Costa (Syn. *Macrolophus caliginosus* Wagner) (Het.: Miridae) in unheated tomato greenhouses of northern Spain through the winter. However, when *Dittrichia viscosa* (L.) W. Greuter (Asteraceae) was used as a reservoir for *M. melanotoma*, the migration to tomato was low, even at high population densities (Arnó *et al.* 2005). *Malacocoris chlorizans* Panzer (Het.: Miridae) is very common in hazel and colonises orchards, but it is not known why it ignores nearby alder, where food would be plentiful (Gange & Llewelling 1985).

The cutting of pumpkin was expected to force *Dicyphus* to move and establish in nearby tomato plants. However, it took a long time for *Dicyphus* to leave pumpkin, and migration to tomato was much lower than expected. Curiously, in the weeks following the cutting of pumpkin, sticky traps on the outside walls of the compartments where pumpkin plants were left intact achieved a significantly higher number of *Dicyphus* captures. The same kind of behaviour was observed in *D. hesperus*, the migration of which from mullein to pepper (*Capsicum annuum* L. (Solanaceae)) plants was very low when plants were cut (Gillespie & Sanchez, unpublished data). In contrast, when mullein was used as a companion plant in tomato settings, *D. hesperus* moved easily to tomato plants as whitefly abundance increased (Sanchez *et al.* 2003b). Laboratory studies showed that mullein was the most-preferred host species, with intermediate preference for tomato and very low preference for pepper (Sanchez *et al.* 2004); thus, the low population flow to pepper in the field experiments was attributed to host preferences.

Many species of omnivore dicyphine occur at high numbers only on a restricted range of plant species and the few studies carried out so far seem to indicate that they have developed a high preference for plants normally used as their host in nature (Sanchez *et al.* 2004). However, these plant preferences have rarely been taken into account when working with these species in biological control programmes. It is expected that success in the use of insectary plants for maintaining populations of predatory dicyphines within crops and their surroundings, and for crop colonisation, will depend to a great extent on the relative preferences for the alternative hosts.

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CAN EXTRA-FLORAL NECTARIES AND OTHER SUGAR-BASED FOODS FACILITATE PREDATION BY CURSORIAL SPIDERS IN ROW CROP AGROECOSYSTEMS?

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ABSTRACT.

Long-term studies observing sentinel eggs of the pest moths *Helicoverpa zea* and *Spodoptera exigua* in south Texas row crops has shown that cursorial spiders (Anyphaenidae, Miturgidae, Lyniphiidae, and Clubionidae) are important predators of the eggs. The cursorial spiders are the dominant predator of lepidopteran eggs in cotton but are less important in crops that lack extra-floral nectaries, such as corn and soybean suggesting that extra-floral nectaries are important in maintaining predator abundance. Support for this idea was provided by an analysis of spiders subjected to the cold anthrone test that showed that nectarivory occurred frequently in cotton fields. Extra-floral nectaries are a significant resource for many natural enemies but their importance to cursorial spiders is poorly understood. Feeding experiments showed that these spiders consumed a variety of sugars found in extra-floral nectar and honeydew. HPLC analysis of sucrose-fed spiders showed that they readily converted sucrose into its constituent monomers and rapidly assimilated fructose. Furthermore, laboratory diet studies demonstrated that consumption of sugar and other non-prey food resources could significantly improve their survival, growth, and development. Behavioral studies showed that they were stimulated by nectar aroma and could learn to recognize and remember novel aroma cues. In combination, these data suggests that cursorial spiders are adapted for finding and utilizing extra-floral nectar and that their role as biological control agents can be strongly influenced by nectar availability.

INTRODUCTION.

Nocturnal cursorial spiders in the families Anyphaenidae, Clubionidae, Lyniphiidae, and Miturgidae are consistently among the most important predators of lepidopteran eggs in annual crops, particularly cotton, in the lower Rio Grande Valley of Texas (Pfannenstiel 2005, 2008). Mortality of sentinel lepidopteran eggs during the period these spiders were observed in cotton was typically between 30 and 80 percent over a 24 h period. These spider taxa are also potentially important predators of lepidoptera and other pests in cotton in central Texas (Breene *et al.* 1989; Nyffeler *et al.* 1990) and Georgia (Ruberson & Greenstone 1986) and members of these families are important predators in other crops such as apples (Miliczky & Calkins 2002), citrus (Amalin *et al.* 2001; Pfannenstiel & Patt unpub. data) and pecans (Richman 2003). However, relative spider importance varies considerably among crops; in south Texas, they have been consistently more important in cotton than in corn or soybean (Pfannenstiel 2008). The presence of extra-floral nectaries in cotton is a likely explanation for the consistent importance of these spider taxa in that crop.

Extra-floral nectaries are a significant resource for many natural enemies (Wäckers *et al.* 2005). However, the importance of extra-floral nectar to nocturnal cursorial spiders (Anyphaenidae, Miturgidae, Lyniphiidae and Clubionidae), which are common foliage inhabitants, is poorly known. Taylor & Pfannenstiel (2008) demonstrated that a large proportion of the spiders in the Anyphaenidae and Miturgidae collected at night from cotton fields had fed on extra-floral nectar. Nectar availability was also demonstrated to improve survival and reproduction of prey-limited *Cheiracanthium inclusum* (Hentz) (Araneae: Miturgidae) under laboratory conditions (Taylor 2004; Taylor & Pfannenstiel unpub. data). Our laboratory studies are examining the relationship between nectar availability and its effect on spider survival, development and reproduction. Behavioral and feeding studies are being used to explore the ability of the spiders to use chemical cues to locate sources of sugar and to assimilate various types of sugars found in nectar and honeydew.

MATERIALS AND METHODS.

Laboratory studies were used to evaluate the acceptance and assimilation of sugars from nectar and honeydew, the impact of sugar consumption on spider growth and survivorship under varying levels of prey limitation, and the influence of contact and olfactory cues on searching behavior for nectar and prey.

Sugar Acceptance, Assimilation and Support for Development.

Acceptance of nectar and honeydew sugars by cursorial spiders was evaluated by observing feeding by 3rd instar *Hibana futilis* Banks and *Hibana arunda* Platnick (Araneae: Anyphaenidae) and *C. inclusum* on a droplet of 2 M sugar solutions under a dissecting microscope. The sugars evaluated were the nectar sugars sucrose, glucose, and fructose and the honeydew sugars, maltose, melizitose, and raffinose. Individual spiders were placed on a glass Petri dish top with a sugar droplet in its center. To facilitate their transfer to the Petri dish, the spiders were anaesthetized by gentle chilling for 60 sec. to 180 sec. just prior to testing (Patt & Pfannenstiel 2008). Chilling was accomplished by transferring a culture dish with its spider from the incubator into a -12°C freezer. Once anaesthetized, the spiders were immediately transferred from their culture dishes to the arena with an artist's paintbrush. When transferred to the arena, the chilled spiders were placed so that their front tarsi contacted the sugar droplet. Spiders that did not become active within 90 s after transfer to the arena were returned to the incubator. For each spider, acceptance or rejection of a particular test sugar was determined through behavioral observations; and the time to initiation of feeding and total feeding time was measured with a digital stopwatch.

The assimilation of sucrose by *H. futilis* was evaluated using the general technique described above followed by freezing the spiders at 10 min., and 1-, 12-, 24-, 48- and 96-h intervals after feeding. These spiders were then subjected to HPLC analysis to evaluate the types and amounts of sugars present in their bodies at each time interval after feeding (Steppuhn & Wäckers 2004).

The value of sucrose and a sucrose-containing diet for spider development were evaluated using spiders that were placed on a prey limited diet with or without sucrose or the sucrose-containing diet supplement, wheast (a mixture of sucrose, brewers yeast, whey and soy). In this experiment, spiders were provided with either 0, 5 or 15 *H. zea* eggs during each instar. A diet of 5 eggs corresponds to ca. 35% of the eggs fed upon by a spider when provided eggs *ad libitum* prior to their molt from the first- to second instar. A diet of 15 eggs was more than typically consumed before molting to the 2nd instar and corresponds to ca. 68% of the eggs fed upon before molting from second- to third instar when fed *ad libitum* (Pfannenstiel unpub. data). Therefore, spiders provided with 5 eggs were considered *a-priori* to be prey-limited for the entire course of the experiment while spiders provided with 15 eggs became prey-limited after they reached second instar. Newly hatched spiderlings were assigned randomly to one of nine treatments: water alone (no food), 5 eggs alone, 15 eggs alone, sucrose alone, sucrose + 5 eggs, sucrose + 15 eggs, wheast alone, wheast + 5 eggs, or wheast + 15 eggs. Spiders were only given the eggs at the beginning of a stadium and were not provided with additional eggs until and unless they molted to the next stadium. All spiders were provided with water using a 1 cm long section of moistened dental wick. Sucrose or wheast was provided on a plastic cover slip and was changed twice per week. As of this writing, this study is ongoing and results to date will be reported below.

Spider Searching for Nectar and Lepidopteran Eggs.

The ability of spiders to locate and learn to associate odors with nectar sources was evaluated using an inverted Petri dish assay (Patt & Pfannenstiel 2008). A similar assay, with a test arena constructed from the top lid of a glass Petri dish (10 cm diam.), was used to measure the localized searching behavior of immature *H. futilis* following consumption of either prey- or non-prey food. *H. zea* eggs were used as the prey food item and droplets of artificial nectar (12.5% aqueous honey solution) were used as the non-prey food item. Control observations were made of spiders placed on a blank Petri dish without food items. When placed near the center of a Petri dish lid, *H. futilis* tend to move to the dish perimeter and then crawl along the edge (Patt & Pfannenstiel 2008). However, when engaged in area-restricted search, their edge-seeking (thigmotactic?) behavior diminishes. In the observations described below, either *H. zea* eggs or nectar droplets were positioned along the dish edge so that the spiders could quickly locate them and subsequently search the remainder of the dish (Patt & Pfannenstiel, 2008).

For each observation, a spider was presented either with three small nectar droplets (2 µl each) or with three *H. zea* eggs. The food items were placed equidistant from one another along the dish edge. The eggs were anchored to the dish with small droplets of white glue to prevent the spiders from carrying the eggs and confounding analysis of their movement patterns. The spiders remained stationary while feeding on the nectar droplets. The movements were recorded with a video camera and evaluated using the EthoVision behavioral recording and tracking software (v3.0, Noldus Information Technology, Wageningen, the Netherlands). Before testing, spiders were chill anaesthetized as described previously and all observations were conducted in semi-darkness.

Several parameters commonly used to measure path movement patterns (Bell 1991) were used to characterize the spiders' area-restricted search responses. For each observation, the parameters calculated by the software included total distance moved (cm), turning angle (degrees), and velocity (cm/sec.).

RESULTS AND DISCUSSION.

Sugar Acceptance, Assimilation and Support for Development.

Nearly all of the nectar and honeydew sugars were readily accepted by both *Hibana* spp. and *C. inclusum*. The one exception was maltose, which was readily accepted by *Hibana* spp. but usually rejected by *C. inclusum*. Within 24 of consumption, sucrose was broken down into its components, fructose and glucose, and the fructose was completely assimilated and only significant amounts of glucose remained (Fig. 1).

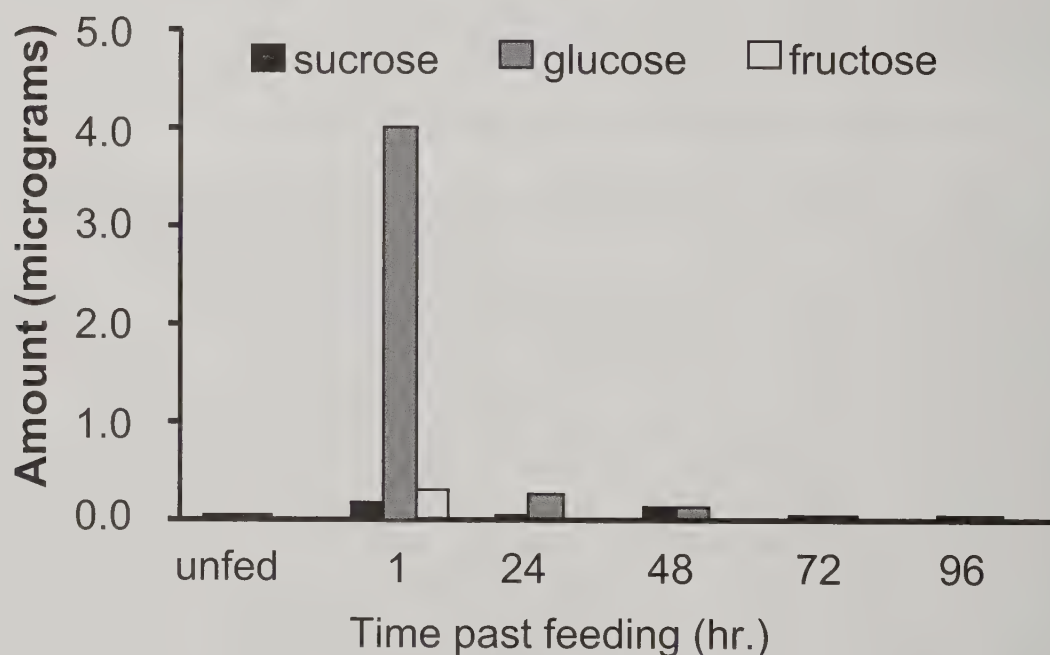


Fig. 1. Presence and amount of sugars in *Hibana futilis* at discrete time intervals following feeding on 2 M sucrose solution (n=3 for each time).

Taylor & Pfannenstiel (2008) demonstrated that, over the course of a two-year survey, between 25 and 55% of female spiders collected from cotton were positive for fructose. Because fructose remains detectable within a spider for only a short time, this suggests that these spiders had fed on extra-floral nectar less than 24 h earlier. It is likely that these spiders were regular consumers of extra-floral nectar.

Provisioning of sucrose or wheast significantly improved the ability of a *H. futilis* to grow on highly prey-restricted diets. When provided with water alone, median spiderling survival was 5 days (range 3 – 8 days) but when provided with sucrose alone, median survival increased to 52 days (range 36 – 67 days). On

severely restricted diets of only 5 *H. zea* eggs per instar, only 37% of spiders molted to the 2nd instar, the addition of sucrose allowed 65% of spiders to reach or exceed the 3rd instar. Spiders provided with 5 eggs per instar plus wheast developed through to at least the 5th instar with 78% reaching or exceeding the 5th instar. When available prey was increased from 5 to 15 eggs per instar with no sugars, 68% reached the third instar and 33% reached the 4th instar. The addition of sucrose to the 15 egg treatment allowed 94% of spiders to reach or exceed the 4th instar and 25% to reach or exceed the 5th instar. Thus far, 94% of the spiders in the 15 egg + wheast treatment have reached or exceeded the 6th instar. One spider in this treatment has developed into an adult male, which reached adulthood after consuming a total of only 90 eggs in addition to wheast. When fed *ad lib* on *H. zea* eggs, *H. futilis* males consumed an average of 610 eggs to reach adulthood (Pfannenstiel unpub. data). When presented to a female *H. futilis*, it successfully courted and copulated with her.

From these data it is apparent that these sucrose and other non-prey resources can provide significant benefit to severely prey limited spiders. Data on *C. inclusum* reared under varying levels of prey limitation (Taylor 2004; Taylor & Pfannenstiel unpub. data) also demonstrated a significant benefit of extra-floral nectar on development and reproduction when prey were limited. The results for wheast are particularly noteworthy because this product could be sprayed in situations where these spiders might be important natural enemies to improve the retention and survival of immigrating spiderlings early in the crop season.

Spider Searching for Nectar and Lepidopteran Eggs.

Spiders that consumed *H. zea* eggs or honey droplets spent a significant amount of time engaged in area-restricted search while spiders in the blank arenas did not display area-restricted search (Fig. 2). No difference was detected in the amount of area-restricted search between individuals in arenas with moth eggs or honey droplets. The degree of turning was also much greater for those spiders exposed to honey droplets or *H. zea* vs. those in the blank arenas. From this study it is not apparent whether *H. futilis* responded more strongly to prey or non-prey resources.

Under all treatments, *H. futilis* demonstrated a capacity for moving quickly over long distances. During observation periods that typically lasted 240 to 300 sec., they covered mean distances of ca. 500 to 600 cm with crawling velocities of up to 2.5 cm/sec. These results are consistent with field observations in which the spider's typical foraging behavior consisted of long bouts of crawling over extensive portions of foliage. This foraging behavior is most likely energetically expensive and frequent consumption of nectar or honeydew may help maintain foraging intensity over time. Studies with this and other similar spiders have demonstrated that they can respond to nectar aromas (Patt & Pfannenstiel 2008) and to solvent extracts of moth scales associated with *H. zea* eggs (Ruiz 2006). Covering large distances while foraging and then responding to local availability or abundance of prey- or non-prey might allow for more efficient discovery of scarce or unevenly distributed resources in heterogeneous environments.

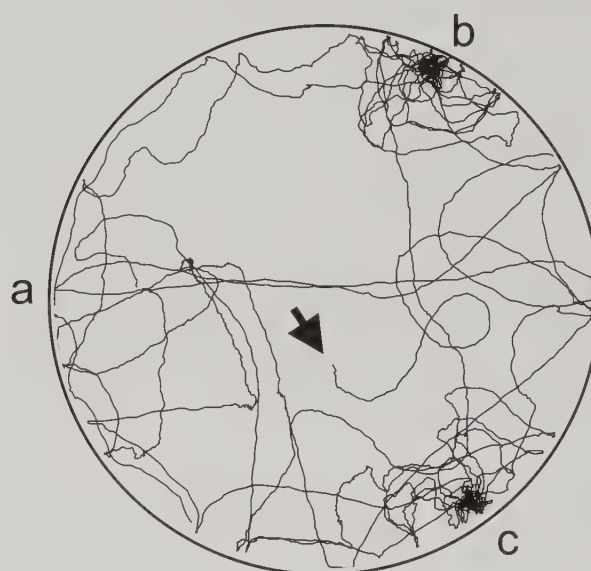


Fig. 2. Motion track of *Hibana futilis* in a Petri dish following consumption of dried sucrose droplets. The position of the droplets on the dish is indicated by letters and the release point is indicated by the arrow.

One possible explanation for the differences in abundance in south Texas annual crops is the presence of extra-floral nectaries in cotton. Recent studies have confirmed that these spiders readily consume extra-floral nectar in cotton (Taylor & Pfannenstiel 2008) and from ongoing studies of sugar digestion by spiders, it is apparent that they feed on these sugars frequently. Location of nectaries can be enhanced by behavioral responses to aroma and plant cues (Patt & Pfannenstiel 2008). The response of these spiders to sugars appears to be similar in type and strength as those to prey lepidopteran eggs (Patt & Pfannenstiel submitted). Characterization of the searching behavior of *H. futilis* has demonstrated that it responds strongly to both sugar sources and to prey eggs. Other studies have demonstrated that feeding on sugars is of significant benefit to spiders when they are prey limited (Taylor 2004, Pfannenstiel & Patt unpub. data).

CONCLUSIONS.

In combination, these data suggests that cursorial spiders are adapted for finding and utilizing extra-floral nectar and that nectar availability significantly increases their role as biological control agents in cotton vs. other crops. Crops with extra-floral nectaries are likely to benefit from enhanced predation pressure on their herbivores. Future studies will examine the role of nectarivory in mediating biological control by cursorial spiders and the potential for improving their performance as biological control agents with resource augmentation. Augmenting crops that do not contain extra-floral nectaries with nectar producing plants or through the use of resource sprays such as wheast may offer significant benefits to these spiders, which, in turn, may increase their impact on crop pests.

ACKNOWLEDGEMENTS.

Sincere thanks go to Robin Taylor for her initial work on nectarivory by spiders in cotton fields and the value of nectar to prey-limited spiders. Her work initiated the path that has lead to these current studies. We also would like to thank Frank De La Fuente, Lupe Vasquez, Orlando Zamora, Allen Ramirez, and Israel Castillo for their dedication and assistance in on various aspects of these studies.

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DIET REQUIREMENTS OF A GENERALIST PREDATOR

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ABSTRACT.

Anthocoris nemorum is among the most important predatory bugs in apple orchards. Research on the dietary requirements of this beneficial insect can provide insights useful for conservation biological control and for developing a rearing method for this pest. An experiment was conducted to assess the dietary quality of *A. nemorum* prey, represented by *Rhopalosiphum padi* for aphids, *Sitotroga cerealella* for Lepidopteran eggs, sucrose and pollen for supplementary plant food and alternative prey represented by Collembola. Diets were provided singly and in combinations. A starvation treatment served as a control. The hypothesis was that because of differences in food quality, diets would affect survival and development differently. Survival differed significantly among the diets, with best survival on mixed diets. No anthocorids survived until the adult stage on the diet of sucrose + pollen. Though a diet of *S. cerealella* results in development and survival not differing significantly from mixed diets, the resulting adult weight and fecundity was significantly lower, showing that anthocorids thrive better when additional prey is supplied. Sucrose + pollen has not previously been tested for their dietary value to *A. nemorum*. Some nymphs survived and developed to intermediate instars on a pure plant diet of sucrose + pollen, stressing the potential value of flowers and/or honeydew for the survival of anthocorids in periods when prey is scarce.

INTRODUCTION.

Anthocoris nemorum (L.) (Hemiptera: Anthocoridae) overwinters in the adult stage and is active as soon as weather permits. Anthocorids are usually found on leaves or branches of trees or in the surrounding vegetation, actively hunting for nymphs of aphids, psyllids, Lepidopteran eggs, and spider mites (Solomon *et al.* 2000). Across Northern Europe, spiders and predatory bugs are the most important early predators in pome fruit. Predatory bugs could make a significant contribution to a reduction in the number of aphids, depending on if a high density of predators is present in the field early in the cropping season (Symondson *et al.* 2002). A community of alternative prey would help to support a high density of anthocorids in the field (Solomon, 1982). The dietary value of the alternative prey would in turn determine its role in maintaining a high population of *A. nemorum* early in the cropping season. An experiment was designed to assess the dietary quality of the most common *A. nemorum* prey in apple, represented by *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) for aphids, *Sitotroga cerealella* (Olivier) (Lepidoptera: Pyralidae) for lepidopteran eggs, sucrose + pollen for supplementary plant food and Collembola. Collembola have often been put forward as an important prey for spiders and carabids (Bilde *et al.* 2000; Sigsgaard *et al.* 2001) and are numerous in trees in early spring, especially older trees. Survival, development, longevity, and fecundity were used as measures of prey quality of *S. cerealella* eggs, aphids, Collembola, sucrose + pollen and mixed diets on *A. nemorum*.

MATERIALS AND METHODS.

Anthocorids and Prey Species, Pollen and Sucrose.

To obtain young nymphs for the experiment, adult female *A. nemorum* were field collected around the Royal Veterinary and Agricultural University (KVL) in Frederiksberg and its experimental orchard in Høje Taastrup. The culture of *R. padi* was based on a colony obtained from the biocontrol company 'Borregaard Bioplant'. They were reared in plexiglass cabinets on potted wheat. *Foliosoma fimentaria* (L.) (Collembola: Isotomidae) was maintained on cowdung compost fed bakers dry yeast at room temperature. Sucrose was used as a surrogate for nectar. Fresh, frozen, bee pollen, principally from fruit trees, was used.

Experimental Conditions.

Anthocorids were reared from field collected females. Eggs were kept in Petri dishes for emergence of immature nymphs. After hatching, allowing 12-24 h for the nymphs' cuticle to harden for handling, nymphs were transferred to medicine cups with a netted lid. Prey was fully replenished every second day, ensuring prey in excess of demand. Water was provided daily or when needed on paper gauze for the nymphs to drink and to maintain humidity. Rearing and experiments were performed at 22°C day and 12°C night with 20L:4D approximating Danish mid-summer conditions with few dark hours.

Diets.

A control treatment of no food, four different single diets and four mixed diets were offered to 5-21 nymphs in each treatment: (1) *S. cerealella* eggs (> 5 mg daily), (2) *F. fimentaria* (six every second day gradually increasing up to 20) (3) 2nd-3rd instar *R. padi* (six every second day gradually increasing up to 10), (4) Sucrose (1 M) + fruit tree pollen (at least 2 pollen grains), (5) *S. cerealella* eggs + *F. fimentaria* (6) *S. cerealella* eggs + *R. padi*, (7) *S. cerealella* eggs + sucrose + pollen, (8) A mix of *S. cerealella* + *R. padi* + *F. fimentaria* + sucrose + pollen.

All the nymphs used in the experiment emerged within three weeks. Nymphs from the same mother were assigned systematically across treatments. Moulting was recorded daily. Controls with the same prey combinations but without *A. nemorum* were used to adjust for control mortality of prey. When mature, 1-2 d old, females were kept with a male for 2-3 days for mating, before the males were removed. Females were kept in Petri dishes on the same diet they received as nymphs. The production of eggs was recorded every second to third day. When adult anthocorids died, they were kept frozen. Width of the thorax as well as total body length were measured with an ocular micrometer under a binocular microscope.

Data Analysis.

Data were analysed for normality and homogeneity of variances. When data were normally distributed, they were analysed by ANOVA. In cases where data were not normally distributed and it was not possible to transform the data so that they would meet the requirements for a parametric analysis, they were analysed using the nonparametric Wilcoxon and Kruskal-Wallis tests (SAS Institute 2003). Survival

distributions were analysed with a nonparametric analysis for survival distributions, LIFETEST-Procedure (SAS Institute 2003).

Food consumption in terms of number of aphids consumed was calculated as the difference between the number of aphids introduced into each cage and the number recovered the following day (alive and dead without visible signs of feeding). The number of dead aphids without visible signs of feeding was compared with prey mortality in a control check without *A. nemorum*. The biomass consumed was computed by multiplying the number of prey consumed by the mean dry weight of an individual of that species and, in the case of the mixed diet, calculating the total weight consumed.

RESULTS.

Survival.

Anthocorids that matured did so after four moults on all adequate diets. Survival distributions for immatures showed the longest survival times in days on the *S. cerealella* and the mixed diets with *S. cerealella*, and significantly shorter survival times on the pure diets of aphids and collembola, on pollen + sucrose and shortest in the control (no food). All anthocorids in the starvation control died before the first moult. No anthocorids on the Collembola diet or on the sucrose and pollen diet survived to the adult stage, whereas most anthocorids on the *S. cerealella* diets, the Collembola diet and the aphid diet survived to the adult stage (Fig. 1).

Development Time.

Diet substantially influenced the development time of the nymphs. Development times for 2nd, 3rd, and 4th-instar nymphs were not significantly different for the diets which included *S. cerealella* eggs or the aphid diet. The nymphal instars fed the diet of sucrose + pollen took the longest to develop. Nymphs on the sucrose + pollen and the Collembola diets, did not survive beyond the 4th instar, therefore, comparisons of development times to the 5th instar and the adult stages were restricted to comparisons between *S. cerealella*, aphid and mixed diets. There was a significant difference between development times to the adult stage. Development to the adult stage took longest on the diet of aphids (mean \pm SE, 29.7 \pm 1.2 days), being significantly longer than on the *S. cerealella* diet (25.9 \pm 0.5 days) ($P < 0.003$) and the mixed diet (25.5 \pm 0.4 days), which were not significantly different ($P < 0.08$).

Adult Weight and Fecundity.

Adults developed on the *S. cerealella*, aphid (*R. padi*) and on mixed diets. Highest weight was obtained on the mixed diet (22 \pm 3 mg), though not significantly different from the *S. cerealella* + collembola diet (17 \pm 1 mg), intermediate on the *S. cerealella* + sucrose + pollen diet and the *S. cerealella* + aphid diet (11 \pm 2 mg and 7 \pm 1 mg, respectively) and lowest on *S. cerealella* + aphid diet (2 \pm 0.1 mg and 3.4 \pm 0.7 mg, respectively) ($P < 0.0001$). Female fecundity was significantly affected by diet (Proc GLM, $P < 0.0009$), with highest fecundity on the mixed diet and the diet with *S. cerealella* + aphids (27.4 \pm 6.9 and 19.8 \pm 10.4 eggs, respectively). Fecundity on the aphid diet was intermediate (6.6 \pm 6.6 eggs), while mean fecundity on diets of *S.*

cerealella and *S. cerealella* + sucrose + pollen were both below one egg per female. For practical reasons, fecundity of *A. nemorum* reared on *S. cerealella* + Collembola was not assessed.

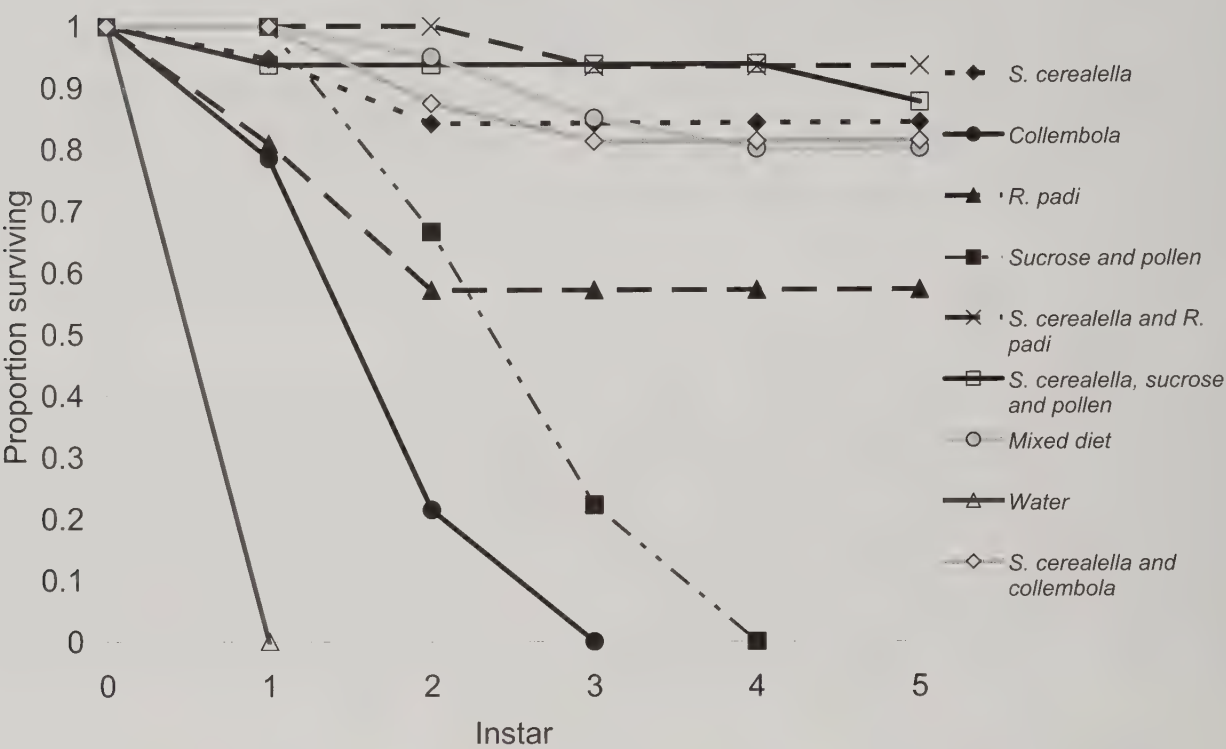


Fig. 1. Diet-dependent survival of nymphs

DISCUSSION.

Across most fitness parameters the dietary value of a pure *S. cerealella* diet was low, leading to a low adult weight and low fecundity. *A. nemorum* were not able to complete development on a pure diet of pollen + nectar, but nymphs were nonetheless able to survive to the third instar on the diet. A pure aphid diet was also not optimal, leading to small adults and only intermediate fecundity. Mixed diets resulted in better survival. The fully mixed diet yielded the highest adult weight, though not significantly higher than the diet of *S. cerealella* mixed with Collembola. Thus, though a pure diet of Collembola may not be optimal, Collembola in the diet positively affects development, survival and adult weight. Likewise, addition of sucrose + pollen to a *S. cerealella* diet, led to higher adult weight.

For early nymphal instars, some mortality was found on the aphid diet. Early mortality on the aphid diet could be attributed to *A. nemorum* difficulties in handling nymphs, though it was provided with the smallest *R. padi* nymphs. Since Collembola were observed to defend themselves against young *A. nemorum*, Collembola were freeze killed before fed to the *A. nemorum*. Despite of this no *A. nemorum* nymphs survived to third instar on this diet.

The study shows that a 'flower-diet' of pollen + nectar can be important for nymphal survival in the absence of aphid prey. It also shows that Collembola, traditionally considered 'neutral' in a pest-beneficial system, can provide an important dietary supplement for *A. nemorum*. Similar results have been obtained for spiders, though the value of different Collembola species may vary considerably (Toft & Wise 1999; Bilde *et al.* 2000; Sigsgaard *et al.* 2001).

CONCLUSIONS.

Anthocoris nemorum is an important aphid predator and can consume many aphids, and it shows preference for aphids over other prey (Sigsgaard 2005). In addition to providing an alternative food source when the number of aphids are low, collembola, pollen and nectar and lepidopteran eggs may also provide anthocorids with a high quality dietary input necessary for them to realize their full predatory potential.

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SESSION 10

THE ROLE OF THEORY IN GREENHOUSE BIOLOGICAL CONTROL

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Field research in biological control is often intrinsically linked with laboratory work, researchers either verifying results from the field or using the laboratory to test proof of concept. This session was proposed to explore the close link between theoretical understanding of systems and how greenhouse environments may augment and assist this understanding. The authors seek to address and build on the idea that, within a closed system such as a commercial greenhouse, the role of theory plays an important part in developing successful biological control strategies.

Theoretical investigations such as population dynamics, are an integral component of any biological control system, although greenhouses present a unique opportunity in managing, or being able to predict, environmental conditions that could influence the efficacy of biological control agents. Session 10 will examine applications of biological control that are unique to this growing environment, the steps in theoretical development of these strategies and innovations, and open discussion on how understanding the components of a closed system aids in the development of successful biological control strategies.

IMPORTANCE OF THEORY IN THE BIOLOGICAL CONTROL OF APHIDS IN GREENHOUSES

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ABSTRACT.

A relatively diverse suite of parasitoids and predators are commonly used for biological control of aphids in sweet pepper crops in greenhouses in many parts of the world. Because of the clonal nature of reproduction in aphids, individual variation may result in the invasion of phenotypes in greenhouses that can cause failures in biological control. In British Columbia, Canada, biological control of green peach aphid, *Myzus persicae* has failed in pepper greenhouses due to the presence of a specific phenotype of this aphid. Research toward developing stable approaches to biological control of this phenotype can benefit from the application of ecological theory to various aspects of the problem. A specific case is explored where application of population dynamics theory is linked with an economic model. This approach can furnish a theoretical optimal release rate based on predicted impacts on hosts and costs for agents that can then be used as a starting point for investigations of the effect of release rates on populations. Even in as simple a case as this, it is clear that ecological theory can provide a necessary starting point for biological control research.

INTRODUCTION.

Biological control of insect pests has long been regarded as an application of the principles of ecology (e.g., Kelleher 1977; DeBach 1974,). If this is true, then the body of theory encompassed by the field of ecology should be important to the development and application of biological control of pest insects. In other words, theory should have some predictive value in the development and conduct of biological control programs, and should enable researchers to not only understand the outcomes of actions, but to compare the outcomes of potential actions, and predict their impacts. This viewpoint is contrasted against one which holds that trial and error is the most parsimonious approach to biological control – that is, programs should be conducted iteratively until the best approach (for example natural enemy, or release density or timing), is found (DeBach 1974; van Lenteren 1980). A great deal has been written regarding biological control as applied ecology in recent years, and advances in ecological theory and methodology have swung the pendulum strongly in favor of a scientific approach to biological control using ecological theory to guide the development and practice of biological control. Recent seminal works that explore aspects of the ecology and science of biological control include Wajnberg *et al.* (2008), Brodeur & Boivin (2006), Hokkanen & Hajek (2003), Agrawal *et al.* (1999), Wäkers *et al.* (2005), Gurr *et al.* (2004), and Bigler *et al.* (2006). The intent of this paper is not to re-visit the “art vs science” controversy in biological control, or to review ecological theory and biological control. The purpose is to

provide a simple demonstration of the use of ecological theory to develop practical approaches to a biological control problem in greenhouse agriculture.

A PRACTICAL PROBLEM.

Phenotypic variation among hosts and natural enemies can be important in the outcomes of biological control (Foster *et al.* 2003; Henry *et al.* 2006, 2008). Phenotypic variation in the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), has recently become a biological control problem in greenhouse pepper farms in British Columbia (Gillespie *et al. in press*). Growers experienced difficulty controlling *M. persicae* with parasitoids and predators in greenhouse pepper crops, and populations increased to levels where the growing tips of plants were killed. Observations suggested that the difficulty was associated with a particular phenotype, which was noticeably darker green in color than other phenotypes. In the course of these observations, a second distinct phenotype was identified, which was light red in all its developmental stages. Clones were established from these phenotypes, and from a third phenotype (light green) selected from a population under biological control on greenhouse pepper plants. The biology of these clones was investigated with respect to reproduction, susceptibility to attack by *Aphidius colemani* Viereck (Hymenoptera: Aphididae) and pesticide resistance, and the results are summarized in Table 1. The dark-green clone, from the troublesome, dark-green phenotype, was found to increase in numbers at a greater rate, and was less susceptible to parasitoids than the other two clones examined. This clone also had lower pesticide resistance than the other two clones. In long-term cage trials in the presence of the parasitoid *A. colemani*, this dark-green clone also increased to higher numbers and took longer to control than the red or light-green clones. These findings are similar to those of other researchers who have investigated clonal diversity in *M. persicae* (Foster *et al.* 2003).

Table 1. Summary of phenotypic responses of three clonal lines of *Myzus persicae* investigated by Gillespie *et al. (in press)*.

Clone	Relative reproduction	Susceptibility to parasitoids	Pesticide Resistance
Light Green	Low	High	High
Red	Medium	High	Moderate
Dark Green	High	Low	Low

Biological control in greenhouse pepper crops in British Columbia has changed as a result of the presence of the dark-green phenotype of *M. persicae*. Growers are less willing to tolerate increasing populations of *M. persicae*, and more readily use pesticides, particularly neonicotinoids such as imidicloprid. These in turn impact natural enemies used for biological control of other pests in pepper crops. Constant, low level introductions of predatory midges, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) in advance of the appearance of aphids in the crop, and increased release numbers of parasitoids in the presence of increasing aphid numbers have been considered as approaches to biological control of this phenotype, but it is not clear if these are effective, or economic for growers.

OUR THEORETICAL ROOTS ARE SHOWING.

The problem with ecological theory, in general, is that there is no particular shortage of it. Ecological theory which is relevant to biological control spans the breadth of the field: evolutionary ecology, invasion ecology, community ecology, population ecology, behavioural ecology, chemical ecology, molecular ecology and more. Even for very specific problems such as the problematic phenotype of *M. persicae*, all of the above fields are relevant. In the context of invasion ecology, we should want to know the source of the clone, and how it is transmitted from greenhouse to greenhouse. Greenhouses can be thought of as islands isolated from the surrounding ecosystem. Island biogeography might provide a useful general theoretical framework for this question (MacArthur & Wilson 1967), and theory surrounding invasions and invasive species would provide some insights into the investigation of movement and pathways of invasion (Elton 1958; Ehler 1998; Fagan *et al.* 2002). Evolutionary ecology has great relevance to the problem, ranging from the genetic diversity of *M. persicae*, in general (e.g. Foster *et al.* 2003), to how one might capitalize on plasticity and local adaptation of natural enemies to a particular phenotype (Henry *et al.* 2008). Competition among clones on plants, or among natural enemies on hosts are obviously relevant (community ecology), as are factors affecting population growth (population ecology) and interactions of the pest genome with the plant genome, affecting such functions as natural enemy signaling (chemical and molecular ecology).

One of the key features of biological control in greenhouses is that it is based on the purchase and release of agents. The costs of agents should be competitive with alternative technologies, such as chemical insecticides, and affordable and effective in the overall milieu of an IPM program. With crop production costs spiraling and research dollars decreasing, biological control researchers are frequently challenged by management and grower-operated funding agencies to ensure that research is directed toward developing technology and approaches that are not only effective, but are affordable.

DEVELOPING A BIOLOGICAL CONTROL APPROACH WITH THEORY.

One of three phenotypes of *M. persicae* studied by Gillespie *et al.* (*in press*) is non-susceptible to the parasitoid used by the greenhouse industry for biological control of *M. persicae*. Despite this, biological control of this pest should be possible. Given that biological control is expensive to develop (but not nearly as expensive as developing new insecticides), ecological theory should help decide which biological control strategies to explore. There is a tremendous amount of theory in population ecology that is relevant to predicting the most appropriate strategy for introducing natural enemies. Unfortunately, much of this theory is not particularly accessible to biological control researchers without good mathematical and computing skills.

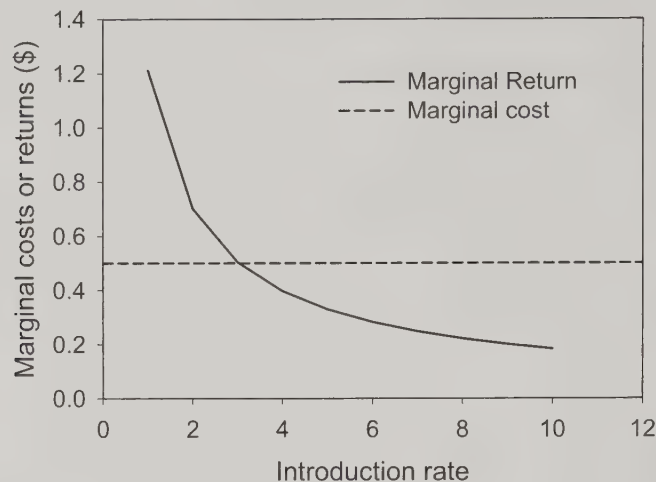


Fig. 1. Graphical marginal incremental analysis requires placing the cost and benefit of an action in a common currency. Actions that place the per capita incremental return above the per capita cost of the action return a net profit.

Greenhouse growers expect to realize a net monetary gain from releases of biological control agents. The cost of an agent should therefore not exceed the value it returns to the grower, but even more so, there should be an optimal investment that maximizes profit from inundation. Roitberg (2004) applied graphical marginal incremental analysis to the problem of determining costs and returns associated with mass rearing natural enemies. Such an approach could also be applied to evaluate biological control agents in release programs. In other words, ecological theory could be applied to an economic problem to estimate the theoretical optimum release rates, which then supplies a starting point to investigate the efficacy of different release rates. Here, the marginal cost for a biological control agent is the incremental per capita cost of the agent, and the marginal return is the incremental per capita value of that agent to a grower, with both cost and returns expressed in a common currency, as in Fig 1. In this diagram, the cost is \$0.50 per agent and does not change with release rate. The per capita value of additional agents in terms of dollars gained, decreases across a range of introduction rates. An introduction rate of about 3 agents is optimum, in terms of the return on investment. Additional agents actually cost more than they return, and fewer agents than the optimum does not maximize investment.

How might we apply such an analysis to evaluate potential research directions and agent introduction strategies for inundative releases of biological control agents against green peach aphids in greenhouses? One approach might be to ask what range of introduction rates should be considered in experiments. Answering this question requires a few simple parameters to describe population growth rates of pests and biological control agents, and the per capita costs of the pest and agent.

Population growth rates can be modeled by a number of equations that can be plotted across a range of values using simple spreadsheet functions. Central to most of these equations is the net reproductive rate, r , which is simply the number of births minus the number of deaths occurring in an interval. There are a number of different approaches that can be used to determine r for a species. In order to illustrate the

approach, I used Euler's exact r to estimate net reproductive rate for the three populations of aphids described in Gillespie *et al.* (*in press*).

$$1 = \sum_{x=0}^{\infty} e^{-rx} l x m x$$

EQN. 1.

The approach uses a simple iterative spreadsheet goal-seeking function to fit r to the known population growth of the three aphid clones over 12 days, where mortality and reproduction ($l x$ and $m x$, respectively) are known for each time step, x . (Table 2).

Table 2. Net reproductive rate and sensitivity to crowding calculated for three clones of *M. persicae* using Euler's exact R (EQN. 1) and iterative goal seeking in a spreadsheet program to fit field data for *M. perscicae* described in Gillespie *et al.* (*in press*).

Clone	R (Net reproductive rate)	c (sensitivity to crowding)
Light Green	7.26	0.129
Red	7.32	0.185
Dark Green	7.42	0.215

The estimated value of r can then be used in population growth equations, such as the form of the Lotka-Volterra equations used by Pimm (2002) to explore food webs and population dynamics in communities, with some slight modifications.

$$N_{t+\Delta t} = N_t + \Delta N (R * (1 - (N_t / K)^c - d_n * P$$

EQN. 2

and

$$P_{t+\Delta t} = P_t + \Delta P (d_p - b * N_t)$$

EQN. 3

In these equations, N and P represent prey and predators, respectively, and K is the carrying capacity. Predator growth rates are strictly determined by prey availability. The term Δ represents the size of the time step, R is the reproductive rate of the prey, and d_n and d_p are the death rates of the prey and predator, respectively. The death rate of the prey is a function of the numbers of predators present, and the birth rate of the predator, b , is a function of the numbers of prey available. The term $1-(N/K)^c$ decrements the net reproductive rate as the population approaches K , as a function of c , the sensitivity to crowding. Sensitivity to crowding therefore decreases with increasing values of c . The term c was determined for the three aphid strains by fitting equation 2 to the observed population growth of the three aphid strains over 12 days (Gillespie *et al.* *in press*) with r as in Table 2. The three lines presented in Fig. 2 thus represent three aphid strains, each with a slightly different net reproductive rate and sensitivity to crowding. Other equation parameters were time step = 0.1, $K = 1000$, and starting $N = 10$. Great caution should be used in interpreting the lines, as they do not model true growth rates or mimic real populations. They simply provide three different datasets from which some ideas in biological control of aphids can be explored. Applying the same parameters to the predator equation for each aphid

yields somewhat different dynamics which reflect, to some degree, growers' experience with the three strains (Fig. 3). The dark green aphid goes into outbreak quickly, and natural enemies reach high numbers before numbers decrease. In contrast, the light green aphid goes into outbreak more slowly, and comparatively fewer natural enemies are generated.

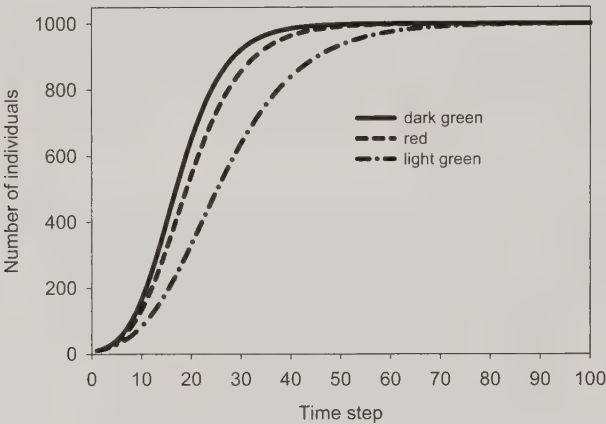


Fig. 2. Exponential growth in aphid populations differing in net reproductive rate and sensitivity to crowding in the absence of predators.

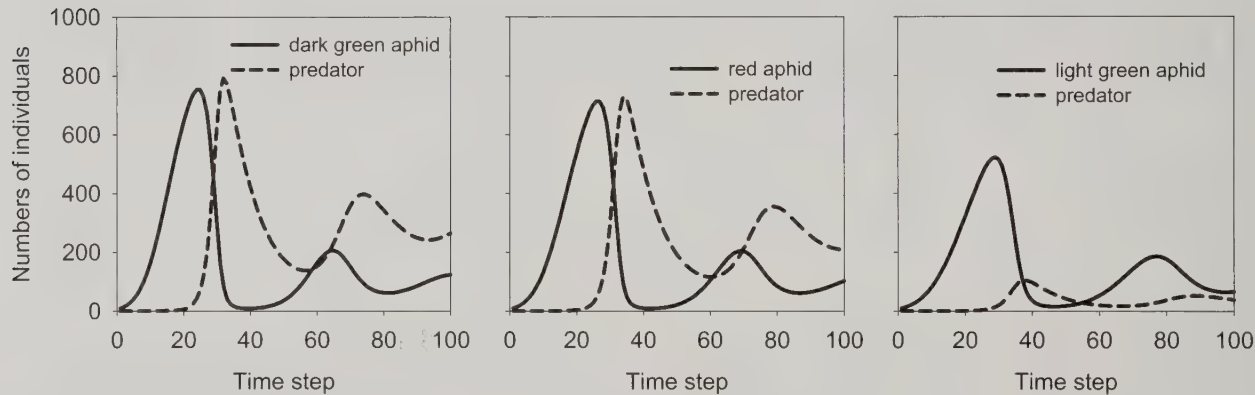


Fig. 3. Population growth of aphid and parasitoid populations following introduction of a natural enemy at 1 per 100 aphids for three clones with different reproductive rates and sensitivity to crowding.

In order to illustrate the approach, I have used the cost of a release of *A. aphidimyza*, at approximately \$38.00 per 1000 adults, to generate a somewhat realistic per capita incremental marginal cost of approximately \$0.04. In order to do a graphical marginal incremental analysis, we need to express the value of releases in the same terms and currency. A pepper leaf can hold approximately 1000 aphids, which coincidentally is K used in the equations above, and it may help to think of the numbers as average numbers per leaf through the greenhouse. Similarly, rather than thinking of the time steps as an abstract 0.1, they can be thought of as units of 1 day. A grower allowing 1000 aphids per leaf to persist in the greenhouse for 50 days would accumulate 50,000 aphid-days per leaf and would certainly experience 100% yield loss. Greenhouse peppers produce about 25 kg per m^2 , at a value of \$4.00 per

kg, at a planting density of 4 plants per m². There are roughly 100 large leaves on a pepper plant that has begun to produce fruit, and this remains relatively constant through the season. Fifty days represents approximately 1/6 of a growing season. Therefore the value of the yield consumed by one aphid-day is \$0.000000835 (\$25.00 x 0.167)/(1000 aphids per leaf x 50 days x 100 leaves). We now have a marginal cost and can calculate the marginal incremental return using the reduction of aphid days achieved by the application of natural enemies at different initial rates from equations 2 and 3 over the initial 50 time steps of the model (Fig. 4).

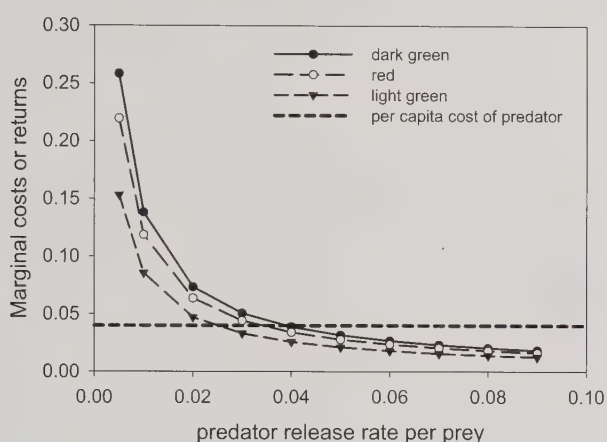


Fig. 4. Graphical marginal incremental analysis of the costs and returns of biological control using a predator against each of three different aphid clones with different population growth characteristics.

At an incremental per capita cost of \$0.04 per predator, the incremental marginal return for the light green aphid intersects the marginal cost at a release rate of 0.025 predators per aphid. Introducing at a rate higher than 2.5 predators per 100 aphids would not return more in yield (through aphid reduction) than the cost of the predators over 50 days. Similarly, the marginal return for the dark green and red strains intersects the marginal cost at around 0.04 predators per aphid, and introducing at rate above 4 predators per 100 aphids would not provide positive incremental economic benefits. These calculations should not be used to determine release rates for biological control agents – that is not their purpose, since they do not account for a host of biotic, abiotic and economic variables. They do however; provide some guidelines for appropriate ranges for experimentation. At 40 predators per 1000 aphids, assuming 10 aphids per leaf at introduction and 100 leaves on a plant, treating a hectare of greenhouse would require 0.004 predators per aphid*1000 aphids per plant*40,000 plants per hectare*\$40.00/1000 predators = \$6,400.00 per ha. The analysis indicates a strong benefit over cost for release rates at or below 4 predators per 100 hosts for an aphid with population growth characteristics similar to the dark green aphid. This might therefore be a useful starting point for experiments on effects of release rates on control. Gilkeson & Hill (1987) found that releases of *A. aphidimyza* against *M. persicae* were ineffective at 1:100 aphids and 1:50 but were effective at 1:6 aphids. However, Hofsvang & Hagvar (1982) found ratios of 1:10 aphids to 1:50 aphids to provide satisfactory control. Van Steenis & El-Khawass (1996) found that release rates of 1 *A. colemani* per 6 *A. gossypii* provided control, but that release rates below this did not. Tremblay (1974) found releases of 1 *A. matricariae* per 50 *M. persicae* provided control on eggplant,

but releases at 1:150 did not. The marginal incremental analysis suggests an optimum release rate in the order of 1:50 aphids for the light green clone, and 1:25 for the dark green clone. The estimates of optimum release rates arrived at by rough application of theory agree very well with those produced by iterative experimentation for at least some natural enemies.

A few *caveats* should be introduced at this point. It is clear that the application of simple theory can be useful for developing experimental direction. One should never, however, make a direct leap from theory to application, even where the theory is so well developed and understood as population dynamics. The analysis presented above has taken liberties with parameterization of the models and estimation of costs and returns. The values for r were estimated from 12 days of population growth only, and c was determined in a circular way, by forcing the equation for population growth to pass through the numbers of aphids observed at 12 days. The values for predator reproduction and impacts on hosts were determined by iteration, and were simply those that didn't cause the models to become unstable across the range of prey introduction rates. The values for economic injury were determined by rough estimation, and refining those estimates through experimentation would certainly change the estimates of the per capita cost of aphids. Moreover, those estimates will be affected by the value of peppers, the cultivar's tolerance to aphid damage, and by other factors affecting production. The discussion has imagined a K of 1000 aphids per leaf, but in these Lotka-Volterra models, K and N do not have units. The spatial scale at which the interactions between the aphid and the parasitoid take place will also have a bearing on the estimations of per capita returns from predator releases. The Lotka-Volterra models are applicable to predators, and to conduct a similar exercise for parasitoids, Nicholson-Bailey models would be appropriate. As pointed out by Roitberg (2004), the per capita value of an agent can vary considerably under different production conditions. This per capita value of the agent to the insectary translates directly into a per capita cost to the grower releasing the agent. It would be rather simple, but somewhat pointless, to continue to describe places where theory and reality disagree in the analysis above. However, with a few simple experiments to properly parameterize the models and place some real values on costs and returns, the models above could be used to explore the effects of release strategies and parasitoid biology on economic outcomes, and to then develop experiments that can test realistic and affordable approaches to biological control, for example, of a problematic phenotype of green peach aphids.

CONCLUSIONS.

The challenge in preparing this article for the session on the importance of theory in biological control in greenhouses has been to avoid two traps. The first was to not resort to simply summarizing the various relevant bodies of theory available in ecology. That has been done far more elegantly and extensively by others. The second was to not present experimental data, then use ecological theory to explain the outcome of the experiment. The first trap allows general prediction without application, and the second, specific explanation without prediction. In applying population dynamics theory to graphical marginal analysis of a biological control problem, I have intended to demonstrate that ecological theory should be used to develop testable predictions about applied biological control problems, and to

suggest that, at least for inundative biological control problems in high-value crops, economic analysis of costs and benefits of biological control is a necessary part of the research program.

ACKNOWLEDGEMENTS.

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THE POTENTIAL USE OF LURES FOR THRIPS BIOLOGICAL CONTROL IN GREENHOUSES: PRACTICE AND THEORY

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ABSTRACT.

Exploiting the response of thrips pest species to odours has long been a goal for improving thrips pest management including biological control. Applications of attractants could include improved monitoring, push-pull (in conjunction with a repellent odour), lure and kill, and lure and infect technologies, and surveillance for invasive organisms. We have recently discovered that 4-pyridyl carbonyl compounds can elicit responses from a range of thrips species (*Thrips tabaci*, *T. major*, *T. obscuratus* and *Frankliniella occidentalis*) in the laboratory, in glasshouses and in open field bioassays. Some of these compounds can increase the trap capture of these thrips species in both commercial greenhouses and broad acre commercial crops where these species are considered pests. However, our understanding of the mechanisms eliciting this response in thrips is still only rudimentary. Greater knowledge of the underlying behavioural mechanisms, including the intrinsic and extrinsic factors that may affect these responses, as well as optimal trap design and configuration, and odour formulation, will be essential if semiochemical-based approaches are to be integrated into thrips management programmes.

INTRODUCTION.

Thrips, including *Frankliniella occidentalis* Pergande, *Thrips tabaci* L. and *T. palmi* Karny (Thysanoptera: Thripidae), are key pests of greenhouses and outdoor crops (Lewis 1997; Kirk & Terry 2003; Cannon *et al.* 2007) because of their ability to damage plants directly through feeding and indirectly through transmission of plant viruses. Biological attributes such as polyphagy, vagility, rapid reproduction, cryptic behaviour and insecticide resistance make them particularly difficult to manage (Mound & Teulon 1995; Morse & Hoddle 2006). Consequently, there has been considerable interest in developing a range of new methods for thrips pest management, including the use of semiochemicals as lures (Koschier 2008).

So far adult thrips lures fall into two main groups: (1) aggregation pheromones identified for *F. occidentalis* (de Kogel & van Deventer 2003; Kirk & Hamilton 2004; Hamilton *et al.* 2005); and (2) kairomone attractant chemicals derived from host plants and related compounds (Kirk 1985; Teulon *et al.* 1993, 2007a; Davidson *et al.* 2008). An alarm pheromone has also been identified from *F. occidentalis* (Teerling *et al.* 1993; MacDonald *et al.* 2002). In this paper we will concentrate on the kairomone lures as these have been the main focus of our research in recent years and there are some examples where such volatiles bring about large increases in thrips capture compared to control treatments (e.g. NZ flower thrips / ethyl nicotinate >100x (Teulon

et al. 1993), *Thrips hawaiiensis* (Morgan) (Thysanoptera: Thripidae) / methyl anthranilate >800x (Murai *et al.* 2000)).

The first report of volatile odours increasing capture of thrips was by Howlett (1914). Since then a range of methods have been used to study thrips (including greenhouse pest species) semiochemicals, including air entrainment, head space analysis, gas chromatography and mass spectrometry (GC-MS), electro-antennography (EAG), and Y-tube olfactometers, flight chambers, and field experiments (outdoors or indoors) (Koschier 2008). Some methods provide no information on the behavioural response of the thrips (e.g. EAG), while others may only measure the response of walking thrips (e.g. Y-tubes), flying thrips (flight chamber) or both (outdoor field bioassays).

A comprehensive review of the volatile compounds that attract adult thrips has recently been completed by Koschier (2008). In this paper we summarise potential practical uses of chemical lures, especially for biological control, and the theoretical understanding of their effects. We use the term lure to describe chemicals that elicit a positive response from thrips.

PRACTICE.

The main semiochemical-based pest management approaches are: mass trapping, lure and kill and mating disruption (El-Sayed *et al.* 2006) and improved monitoring.

Improved Monitoring and Mass Trapping.

Information obtained from coloured sticky boards on the presence/absence and seasonal abundance of thrips in greenhouses is critical for the appropriate timing, choice and quantity of (bio)control agents released for thrips control (Yano 2004). The addition of lures to coloured traps increases trap efficacy (Teulon *et al.* 1993; Davidson *et al.* 2007) and thus makes earlier and more accurate detection possible. The use of lures is likely to be most beneficial when thrips populations are low and traps without lures do not indicate the presence of thrips populations. Currently, two commercial products make claims to attract thrips in glasshouse crops: Thripline^{ams}® based on the *F. occidentalis* aggregation pheromone (www.syngenta-bioline.co.uk/productdocs/html/Thripline.htm) and LUREM-TR® based on a putative kairomone (www.koppert.com/Products_monitoring.LUREM-TR..14727+M52c9fe7ecac.0.html).

The use of semiochemicals for mass trapping thrips has been explored by a number of researchers (e.g. Kawai & Kitamura 1987; Roditakis *et al.* 2002; Natwick *et al.* 2007) but has not become an established practice. An effective mass trapping method would require a very strong lure and likely be effective only in the presence of several other mortality factors such as biocontrol agents.

Lure and Kill (lure and infect, trap crop, push-pull).

Semiochemicals can be used in other ways to lure thrips to a place where they may be killed (either immediately or at a later time), but none of these approaches

have become established practice. Ludwig & Oetting (2002) investigated a 'lure (Lure®) and infect' [*Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae)] approach without any apparent improvement of *F. occidentalis* control as a result of the addition of the lure. Trap crops, using cultivars or other plant species that are more attractive to *F. occidentalis* than the main crop, have been proposed by several researchers (Bennison *et al.* 2001; Warnock & Loughner 2004; Matsuura *et al.* 2006). Bennison *et al.* (2001) also used the thrips lure (*E*)- β -farnesene to enhance the pull of the trap plant. Thrips can be killed on the trap crops by a number of means, including biological control agents. A slight modification of the trap plant approach is 'push-pull' where repellent compounds are used to push the thrips from the crop (Koschier 2008). Proof of concept of this approach was recently demonstrated by van Tol *et al.* (2007a) with *T. tabaci*, ethyl isonicotinate (lure) and *Origanum majorana* L. (Lamiaceae) (repellent).

Mating Disruption.

It is difficult to conceptualise how mating disruption might work with thrips given the inability to find true sex pheromones and thrips' unique biology. In thelytokous thrips species such as *T. tabaci* (Jenser & Szénási 2004) the inability to find a mate is irrelevant as all the populations are entirely female and unmated females produce only females. However, in arrhenotokous thrips species such as *F. occidentalis* (Brødsgaard 1989) unmated females produce males only and the loss of females from such a population should eventually lead to a population crash.

THEORY.

Research on thrips kairomone lures is still at a rudimentary stage. Some important areas for future research that will help greatly in the development of lures for thrips pest management are outlined below.

Host Finding Behaviour.

The response of thrips to colour and odour in experimental bioassays implicates vision and olfaction as important host finding cues for thrips (Brødsgaard 1990; Teulon *et al.* 1999; Smits *et al.* 2000). Just how thrips use these cues and how they are integrated for host finding are still not understood. There have been many studies examining the response of thrips to lures that measure the end process (i.e. number of thrips caught in a trap) without providing any information on the behavioural process(es) that lead to that outcome. Wind-tunnel bioassays (Teulon *et al.* 1999; Berry *et al.* 2006) indicated that the main response of thrips to a colour cue was directed movement toward the cue but when exposed to an odour cue thrips flight was inhibited. Whether these results are representative of thrips behaviour in more natural conditions or whether this was an artefact of the experimental design (e.g. too a high or too low a chemical dose) is still not clear. van Tol *et al.* (2007a) provided a compelling hypothesis for the behavioural responses to odour and visual cues that included a visual dominated landing response followed by odour dominated upwind movement after landing, but this has yet to be verified with additional experiments.

Mechanism of Olfactory Responses.

While some of the known thrips lures are commonly found in thrips host plants (especially flowers) (Teulon *et al.* 1993) and are therefore presumably involved in the natural thrips host plant finding processes, a number of lures, including some that elicit strong thrips responses (e.g. ethyl nicotinate/*Thrips obscuratus* (Teulon *et al.* 1993), ethyl isonicotinate/*Thrips tabaci* (Teulon *et al.* 2007a), are very rare in nature. Either these 'artificial' lures are much more common in nature than we realise (currently below the level of detection) or they mimic 'natural' lures in binding strongly to olfactory receptors. We can speculate on structural factors affecting binding to such receptors, but studies of insect olfactory receptors are at an early stage (Keller & Vosshall 2007) and no thrips receptors have been studied. We have reported a strong relationship between attractant vapour pressure and lure potency for a series of 4-pyridyl carbonyl compounds (Davidson *et al.* 2008), which may be useful for understanding changes in thrips responses in different environments.

Thrips Physiology.

Recently fed thrips show a weaker response to odour cues than starved thrips (Davidson *et al.* 2006), indicating that lures may be more effective against invading thrips than those resident within the crop. In a wind tunnel experiment odour did not affect the percentage of young (3 day post-emergence) or older (10-13 day post emergence) WFT females landing on a visual cue (Davidson *et al.* 2006). However, more older thrips landed on a yellow visual cue than younger thrips, although a similar percentage of either age group flew in the presence of the yellow cue.

Males and females from arrhenotokous species (e.g. *T. obscuratus*) and females from thelytokous species (e.g. *T. tabaci*) both respond strongly to certain lures (Teulon *et al.* 2007a), providing strong evidence that the lures are not sex pheromones.

Species-specific Responses.

Most lures identified provide responses for thrips species belonging to the genera *Frankliniella* and *Thrips* (Koschier 2008). These genera include many flower-inhabiting species that are key plant pests, including glasshouse pest species (Lewis 1997). However, currently there are no reported lures for *T. palmi*. Many thrips lures attract a range of different thrips species (Koschier 2008) but the strength of response to a given lure differs between thrips species. For example, the proportion of *T. obscuratus* caught in traps with ethyl nicotinate (up to 100x cf. controls) is much greater than for other thrips species (Teulon *et al.* 1993, 2007a). Similarly, the proportion of *T. tabaci* caught in traps with ethyl isonicotinate (up to 62x cf. controls) is much greater than of other species (Teulon *et al.* 2007a). This may be because different lures match different preferred natural attractants. Understanding such specific preferences could lead to the development of species-specific lures.

Dose-dependent Responses.

Y-tube olfactometer experiments, which restricts thrips' response to walking to a specific dose of a given volatile compound, have demonstrated dose-dependent

responses to a range of odours (Koschier *et al.* 2000; Davidson *et al.* 2008). For example *para*-anisaldehyde, at selected doses less than 1 μ l, resulted in more western flower thrips females walking up the odour arm, while at 1 μ l, a similar number walked up the clean air or odour-loaded arm (Koschier *et al.* 2000).

Lure Mixtures.

Only simple trials have been undertaken to blend two or more lures to increase the overall attractiveness. For *T. tabaci*, mixtures of *para*-anisaldehyde and methyl isonicotinate proved to be only as strong as the most powerful lure on its own, (i.e. methyl isonicotinate) (Teulon *et al.* 2007b). However, mixing the strongest known lures for *T. obscuratus* (ethyl nicotinate) and for *T. tabaci* (ethyl isonicotinate) resulted in a similar number of each species captured in a single trap with the mixture compared to separate traps for each odour. (Davidson & Teulon unpub. data). Combining the aggregation pheromone (Thripline_{ams}[®]) and the kairomone (LUREM-TR[®]) currently used in greenhouse crops to trap *F. occidentalis* may prove very fruitful as it would seem plausible that they both act on different receptors.

Host Plant Odour Masking.

While the potential for host plant odours to mask the lure odour has not been studied in detail there is some suggestion that lure effectiveness may be influenced by plants. Trap catches of onion thrips are much higher (cf. controls) in grass fields (84x) (a non-host) than in an onion crop (18x) (a host plant) (Davidson *et al. in press*) although other plant/crop specific characteristics may also be important (e.g. host plant colour). Nevertheless, the efficacy of kairomone lures has been demonstrated in a range of greenhouse vegetable and ornamental crops (van Tol *et al.* 2007b).

Greenhouse Conditions.

The efficacy of kairomone lures has also been demonstrated in a range of different countries from Oceania, Europe, North & South America and Africa (Davidson *et al.* 2007; de Kogel & Teulon unpublished data) although the degree of effectiveness may vary. We are currently investigating the ambient conditions (e.g. temperature, humidity, air flow) that may influence the response of thrips to kairomone lures and how this information may be used to optimise thrips management in different conditions.

Effective Lure Spacing.

In outdoor experiments traps with odour lures influenced trap capture of traps without odour lures 10 m away (Teulon *et al.* 2007c). In greenhouses, where there is generally considerably less air movement than outdoors care is needed to ensure odour lures are placed at appropriate distances from each other to maximise efficacy for the range of management techniques that may be developed with thrips lures. For research purposes it is important to realise that odour-baited traps may influence nearby control traps. From a practical point of view it means that odour-baited traps may also increase the efficacy of nearby unbaited traps.

CONCLUSIONS.

Exploiting the response of thrips pest species to odours has strong potential for improving thrips pest management, including biological control. A greater understanding of the behavioural response of thrips to these odours, including the intrinsic and extrinsic factors that may affect these responses, as well as optimal trap design and odour formulation, will be essential if semiochemical-based approaches are to be integrated into thrips management programmes.

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HOW TO ENHANCE PEST CONTROL BY GENERALIST PREDATORY MITES IN GREENHOUSE CROPS

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ABSTRACT.

Generalist predatory mites are increasingly used in greenhouse crops to control small insects and mites. The success of these predators is strongly affected by crop characteristics. Crops that provide non-prey food facilitate predator establishment. Here we review three methods for improving establishment of generalist predatory mites in greenhouse crops based on (1) crop diversity, (2) pest diversity and (3) alternative food. The underlying theory about food web interactions is discussed in order to predict the effect of these methods on biological control. Furthermore, we show preliminary results of experiments in which alternative food was added to enhance predator establishment.

INTRODUCTION.

Integrated pest management has increasingly included generalist predators feeding on multiple prey, particularly predatory mites (Gerson & Weintraub 2007; Sabelis *et al.* 2008). Recently, the generalist predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) has been introduced for controlling both thrips (Messelink *et al.* 2006) and whiteflies (Nomikou *et al.* 2002), with possible side-effects on other pests. This has been a break-through in IPM greenhouse crops, in particular in the Mediterranean area (see www.allaboutswirskii.com). Generalist predators often feed on pollen and can therefore easily establish in crops that produce suitable pollen, such as sweet pepper (Ramakers 1990). However, most greenhouse crops lack such food and predator introduction must be timed to the presence of phytophagous arthropods. As a result, a temporary peak of the pest population has to be tolerated. This is particularly a problem in ornamental crops, where thresholds of damage are extremely low. Growers therefore frequently introduce high doses of predatory mites. Methods for improving establishment and performance of these predatory mites would lower the costs of biological control and improve efficacy. Here, we review three possible methods for pest control using generalist predatory mites in greenhouse crops.

Crop Diversity.

Due to strict measurements concerning hygiene, the use of soil-less substrates and closed greenhouses, most modern greenhouse crops can be considered as highly simplified ecosystems. The lack of biodiversity hampers establishment of many natural enemies. Experimental studies in outdoor crops show that creating crop diversity with strips of flowering plants or habitats that offer shelter can increase natural enemy abundance, diversity and fitness (Landis *et al.* 2000). The question is whether crop diversity can be applied in modern greenhouse crops for improving pest

control. Mixed cropping is applied on a small scale by organic growers of greenhouse vegetables, but without clear advantages for pest control because predators stay close to their favorite crop (Messelink, pers. obs.). Furthermore, the practical and economic disadvantages of mixed cropping are such that its application is simply out of question in most modern greenhouses. A less drastic method to create crop diversity is the use of banker plants. One elegant system that has been developed for predatory mites uses the castor bean plant, *Ricinus communis* L. (Euphorbiaceae). This plant is an excellent banker plant because it produces enormous amounts of pollen and extra-floral nectar (Van Rijn & Tanigoshi 1999a), and positive results have been achieved with it (Ramakers & Voet 1996). *Ricinus* was originally used for the introduction of *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae), but other phytoseiid predatory mite species such as *A. swirskii* and *Euseius ovalis* (Evans) (Acari: Phytoseiidae) can also be easily reared on these plants. Application of banker plants in greenhouses is limited until now, but we consider castor bean plants as a perfect tool for introducing predatory mites in greenhouse crops where they would otherwise establish with difficulty, in particular for those predatory mites that are hard to mass produce and thus expensive. Castor bean plants might play an important role in future introductions of “new” generalist predatory mites.

Pest Diversity.

A second concept for improving pest control by generalist predatory mites is based on pest diversity. The use of generalist predators for biological control can result in predator-mediated interactions among prey species that otherwise might not interact (Janssen *et al.* 1998; Harmon & Andow 2004). If, for example, the density of one prey species increases, the density of the shared predator also increases and, consequently, the second species decreases in abundance. Holt (1977) has called these interactions apparent competition, because it looks as if the two species compete for a shared resource, whereas in fact it is the shared predator that mediates this interaction. The theory of apparent competition is based on equilibrium dynamics, hence, the effect of a shared natural enemy might be different in the short-term, when equilibria have not been reached. Indeed, increases of one prey species can result in short-term satiation of the shared predator, resulting in a negative effect on the control of the second pest (so-called apparent mutualism, Abrams & Matsuda 1996). The duration of these short-term effects clearly depends on the ecology of the predator. Especially for predators with a long reproduction time this negative effect can be serious (Koss & Snyder 2005; Symondson *et al.* 2006). The challenge for biological control is to apply theory on these predator-mediated interactions between pest species in such a way that biological control is enhanced. Two recent studies with generalist predatory mites indeed showed that pest control can be enhanced by predator-mediated indirect pest interactions. The presence of thrips in a cucumber crop resulted in a considerable improvement of the control of whiteflies (Messelink *et al.* 2008). Spider mite control was also much better in the presence of thrips or whiteflies separately, but the best when both thrips and whiteflies were present (Messelink *et al. in prep*). Disruption of biological control through predator satiation occurred when high releases of whiteflies delayed the control of thrips by *A. swirskii* (Messelink & Janssen 2008).

Pest diversity can also be advantageous when natural enemies grow and reproduce better on a diet consisting of several pests than on single pest diets. For

example, lady beetles have been shown to benefit from diets consisting of mixed aphids (Evans *et al.* 1999). Generalist predatory mites also benefit from mixed pest diets; juvenile development of the predatory mite *A. swirskii* was significantly improved on a mix of thrips and whiteflies compared to a single pest diet (Messelink *et al.* 2008). Such mixed diet effects are believed to amplify the effects of apparent competition. With respect to biological control, growers might thus consider allowing or even introducing some kind of pest diversity in order to enhance control. Such releases of pest species are not a totally foreign concept for greenhouse growers. The “pest-in-first” strategy with spider mites to enhance control by *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) has been successfully applied now for many years by sweet pepper growers (Hussey *et al.* 1965). Pest diversity might not only result in predator-mediated interactions, but also in plant-mediated interactions among pest species. The attack of a plant by one pest species can induce resistance mechanisms in the plant, which subsequently slows the development of a second pest species (Karban & Carey 1984). Theory and empirical data about apparent competition, mixed diets and induced plant defences, encourage us to further study the role of pest diversity in biological control and develop practical methods which can be applied by growers.

Alternative Food.

Generalist predatory mites supplement their diet with non-pest food such as pollen and honeydew (McMurtry & Croft 1997). This offers opportunities to improve establishment of these predators by artificially adding alternative food sources. However, it is not immediately obvious that adding alternative food will also result in reduced pest densities. Theory concerning shared predators (discussed above under *Pest diversity*) is also relevant for alternative non-prey food. Alternative food might decrease the per capita consumption of the target pest prey through predator satiation or may even result in the predators switching to the alternative food, which was shown for the mite *Euseius scutalis* (Athias-Henriot) (Acari: Phytoseiidae) when offered a mix of whiteflies and pollen (Nomikou *et al.* 2004). The alternative food source might also be beneficial for the pest species itself, like pollen is for thrips (Hulshof *et al.* 2003). Shared predation theory predicts, however, that alternative food will always reduce equilibrium pest densities as long as the alternative food source has a positive effect on predator reproduction and survival. Thus short-term negative effects of predator satiation, predator switching or even increased pest reproduction do finally not matter, as this will be compensated by predation from a larger predator population due to the predator’s numerical response to the alternative food (Van Baalen *et al.* 2001; Van Rijn *et al.* 2002). The theory is based on long-term equilibrium dynamics, but when dynamics show fluctuations, apparent mutualism will occur at longer time-scales as well (Abrams *et al.* 1998). Because growing seasons for most crops are short, it is thus important to focus on the non-equilibrium dynamics and determine the circumstances under which the addition of alternative food sources will improve pest control.

Table 1. Reported effects of non-prey food on generalist predatory mites (Acari: Phytoseiidae).

Predatory mite species*	food source	Reported effects	Reference
<i>E. hibisci</i> <i>T. limonicus</i>	Yeast hydrolysate + Molasses or sucrose	Increased longevity, oviposition rate ca. 30% of pollen diet	McMurtry & Scriven 1966
<i>E. hibisci</i> <i>I. degenerans</i> <i>T. limonicus</i>	Mixture of honey, yeast, sugar, egg yolk and casein hydrolysate	Oviposition rate 6-51% of oviposition rates on natural diets	Kennett & Hamai 1980
<i>A. swirskii</i>	Mixture of yeast, milk powder, amino acids, vitamins, sugars and antibiotics	Increased longevity, oviposition rate ca. 50% of pollen diet	Abou-Awad <i>et al.</i> 1992
<i>E. ovalis</i>	Paraffin-diet-chips, based on diet of Kenneth and Hamai (1980)	Low oviposition rate, high juvenile mortality, shorter longevity compared to prey	Sih <i>et al.</i> 1993
<i>N. cucumeris</i>	Protein powders based on casein, soya or Deccan grass	Low oviposition rate	Matsuo <i>et al.</i> 2003
<i>I. degenerans</i>	Sterilized flour moth eggs (<i>Ephestia kuehniella</i>)	developmental time slightly higher than on pollen diet	Vantornhout <i>et al.</i> 2004
<i>I. degenerans</i>	Decapsulated brine shrimp cysts (<i>Artemia franciscana</i>)	developmental time comparable to pollen diet	Vantornhout <i>et al.</i> 2004
<i>N. californicus</i>	Honey, sucrose, tryptone, yeast extract and egg yolk	Low oviposition rate, excellent survival (up to 90 days)	Ogawa & Osakabe 2008

* Names of the genera: A. = *Amblyseius*, E. = *Euseius*, I. = *Iphiseius*, N. = *Neoseiulus*, T. = *Typhlodromalus*.

Many studies report that pollen is an excellent alternative food source for predatory mite species (Van Rijn & Tanigoshi 1999b). Other food sources, such as nectar or honeydew, do not allow for predator reproduction when consumed alone (McMurtry & Scriven 1966; Van Rijn & Tanigoshi 1999a). The many studies on pollen however have not resulted in pollen-based products that growers can apply in greenhouses. One simple reason might be that collecting pollen is labour-intensive and thus expensive. An alternative could be to use bee-collected pollen, which are commercially available at low prices. Sprays of bee-collected pollen was shown to increase the numbers of a predatory mite (Ramakers 1995). However, bee-collected pollen is contaminated with free sugars, and this resulted in fungus growth in humid greenhouses. Many artificial food sources other than pollen seem to have potential for enhancing establishment of predatory mites, but were only investigated in the laboratory (Table 1). In this paper we show preliminary results of the effect of non-prey alternative food for enhancing establishment of generalist predatory mites in a greenhouse crop.

MATERIALS AND METHODS.

Alternative Food Sources and Cultures.

Six sources of alternative food were selected: (1) cattail pollen (*Typha latifolia* L. (Typhaceae)), (2) dried and grinded bee-collected pollen of Cistaceae (www.pollen-online.com), (3) a mixture of yeast, glucose and soya powder, (4) *Carpoglyphus lactis* (Linnaeus) (Acari: Carpglyphidae), the sugar mite on which *A. swirskii* is mass produced, (5) *C. lactis* plus a mixture of yeast and glucose and (6) Aminofeed®, a commercial product based on proteins and sugars (3% solution). We measured oviposition of *A. swirskii* in the laboratory and population dynamics in a greenhouse. Predatory mites for the laboratory experiments were reared on cattail pollen; for the greenhouse experiment they were reared on bran and *C. lactis*. For the greenhouse experiment we used chrysanthemum cuttings cv. "Omega Time Pink", which were planted in 12cm diameter pots in peat.

Oviposition.

The reproduction value of the selected alternative food sources was assessed in a laboratory experiment using the method of Van Rijn & Tanigoshi (1999). Young *A. swirskii* females of the same age were placed on black plastic arena's where the food sources were supplied *ad libitum*. The rate of oviposition was scored from day 4 through day 7. Eggs were removed before they hatched in order to avoid cannibalism on larvae. One replicate consisted of 12 females in one arena and each food source was replicated 4 times. The experiments were performed in a climate room at 22°C, 70% RH and a 16:8h L:D regime.

Population Dynamics.

Population development of *A. swirskii* was assessed on a chrysanthemum crop for a 6 week period on plants without or with the selected alternative food sources. The experiment was set up as a block treatment with 3 replicates. Each block consisted of one table and each plot consisted of a group of 12 plants. Contamination between plots was avoided by a barrier of overlapping sticky plates placed around the plants on the table. Tables were supplied by a standard nutrient solution. Plants of 4 weeks old, containing up to 20 leaves, were supplied with the alternative food sources. Aminofeed® was sprayed in a 3% solution in water. The sugar mite *C. lactis* was released at a density of ca. 500/plant. All other food sources were evenly distributed over the plants at a density of 40 mg/plant. This food application was repeated once after 3 weeks. Shortly after the first food application, we released 20 predatory mites of mixed age per plant. Population development of the predatory mites was followed by weekly picking of 12 leaves per plot (1 leaf/plant) and counting the number of mites under a binocular microscope. The average greenhouse temperature during the experiment was 23.5°C and the average RH was 73%.

Statistics.

For both the laboratory and greenhouse experiment, a repeated measures ANOVA with time as the random factor was performed on the data by using Genstat

(Payne *et al.* 2007). Predator densities in the greenhouse experiment and numbers of eggs in the laboratory experiment were log (x+1) transformed. Differences between treatments were tested at the 5% level using Fisher's LSD (Least Significant Difference) test.

RESULTS.

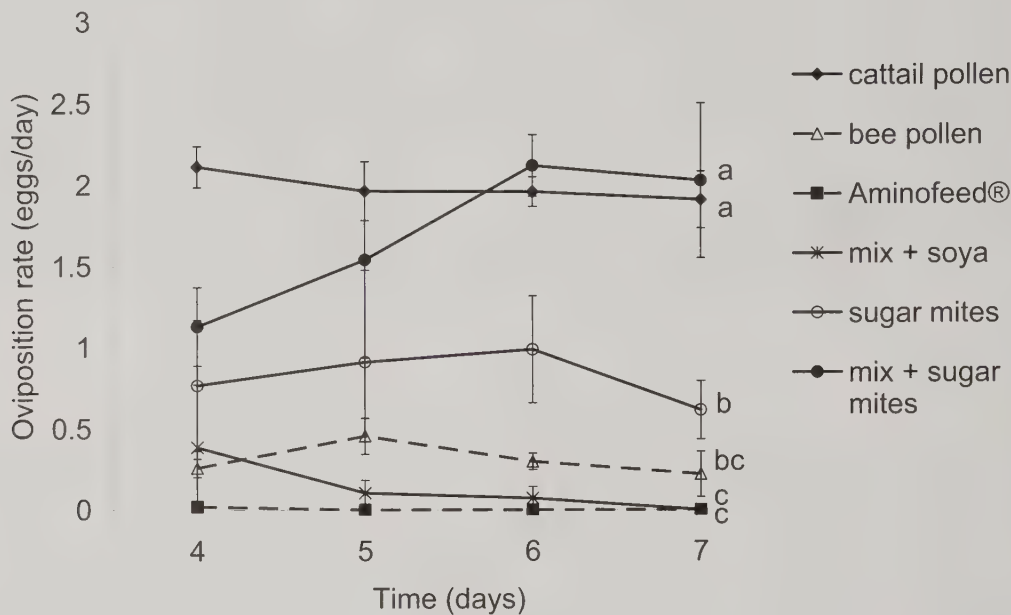


Fig. 1. Effects of alternative food sources on oviposition rates (\pm se) of the predatory mite *A. swirskii* on plastic arenas in the laboratory. Oviposition was measured daily from 4 to 7 days after transition from a diet of cattail pollen to the alternative food source. The mix stands for glucose plus yeast. Food source types followed by the same letters are not statistically different ($P < 0.05$).

There were clear and significant effects of the alternative food sources on both oviposition rate in the laboratory (Fig. 1) ($F_{(5)} = 21.44$; $P < 0.001$) and predator development in the greenhouse experiment (Fig. 2) ($F_{(6)} = 8.65$; $P < 0.001$). Cattail pollen resulted in the highest and most stable oviposition rates in the laboratory (Fig. 1), whereas in the greenhouse experiment the effects on population development were very strong, but short term (Fig. 2). Bee pollen and the diet of glucose, yeast and soya gave low oviposition rates in the laboratory, but significant effects on population establishment in the greenhouse experiment. The addition of yeast and glucose to a diet of sugar mites (*C. lactis*) increased both oviposition and predator densities on the chrysanthemum plants. Predators did not oviposit with Aminofeed® as food source, but population establishment was significantly lower in the greenhouse experiment (Figs. 1, 2).

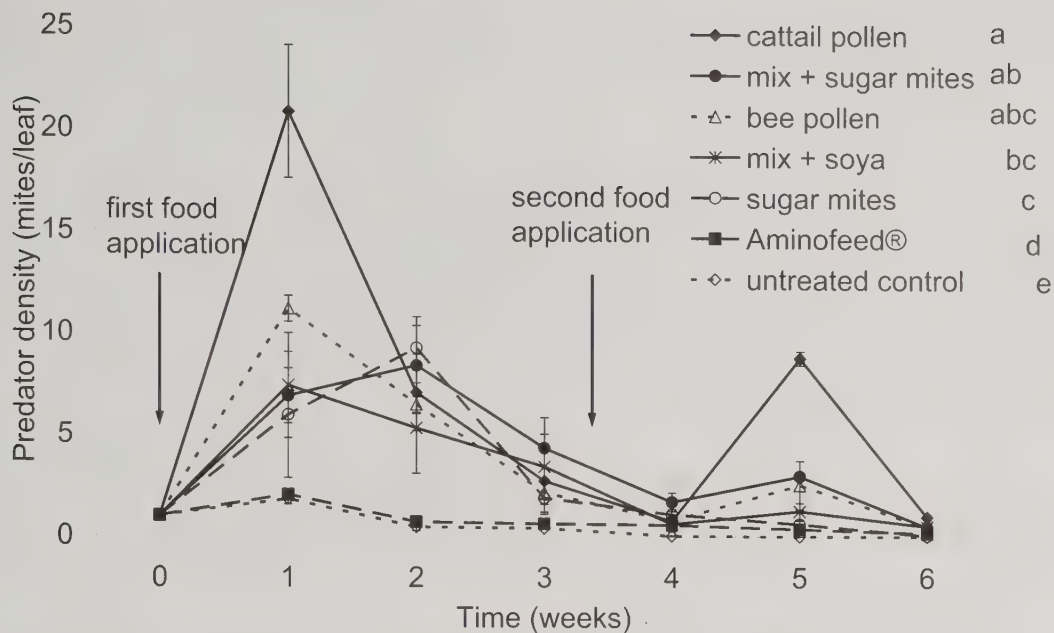


Fig. 2. Effects of the supply of alternative food sources on densities of the predatory mite *A. swirskii* in a non-flowering chrysanthemum crop. Shown are average (\pm se) numbers of predatory mites per chrysanthemum leaf. The mix stands for glucose and yeast. Food source types followed by the same letters are not statistically different ($P < 0.05$).

DISCUSSION.

This study showed that enhancing establishment of generalist predatory mites on plants by adding factitious alternative food sources is a promising method. Many studies have evaluated the nutritional value of such food sources before, but evaluations on the target crop itself are scarce. Recently, Ogawa & Osakabe (2008) suggested using artificial diets to maintain predatory mite populations during periods when herbivore prey are scarce. The results of our experiments are in line with this idea. Bee pollen and a mixture of yeast, glucose and soya were less suitable for predator reproduction, but they look promising for maintaining populations on plants. Cattail pollen was, on the other hand, very suitable for reproduction, but the effects on population development were over time not better than the bee pollen or the mixture of yeast, glucose and soya. Adding sugar mites to plants as alternative food for the predatory mites seems to offer possibilities, as was suggested before by Hoogerbrugge *et al.* (2008). The addition of yeast and glucose significantly improved this effect, probably because both the predatory mites and sugar mites feed on this supplemental food. The laboratory experiment also showed that oviposition on a diet of sugar mites plus yeast and glucose increased with time. A possible explanation is that the young sugar mite stages that were produced on the sugar and yeast diet are more suitable as food for *A. swirskii* than the older stages.

Application of non-prey food sources might not only be important for maintaining predator populations in periods when pests are not present, but also to supplement diets of prey-species in order to increase reproduction. It is known that some invertebrate predators can forage selectively for protein and lipids to redress specific

nutritional imbalances (Mayntz *et al.* 2005). Alternative food sources that supplement prey diets might then even be preferred.

CONCLUSIONS.

Both theory and empirical data about alternative food, offer new possibilities to enhance pest control with generalist predatory mites. Future research should focus on the short-term effects of alternative food (either supplied by banker plants, pest diversity, or factitious food) on biological control of the target pest species.

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USING INSECTS AS A NOVEL APPLICATION STRATEGY FOR THE DELIVERY OF MICROBIAL AGENTS FOR BIOLOGICAL CONTROL OF ARTHROPOD PESTS IN GREENHOUSE CROPS

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ABSTRACT.

Microbial control agents are traditionally applied to crops for arthropod pest control using chemical spray technology. Over the last 10-15 years, pollinator bees have been investigated as vectors for microbial control agents (fungi, viruses and bacteria). This paper reviews the development of the Pollinator Biocontrol Vector Technology and how pollination of crops can be combined with arthropod pest management. This technology can potentially be used for simultaneous pest control and plant disease suppression and extrapolated for use with other insects.

INTRODUCTION.

Traditionally, microbial control agents have been applied to crops for pest control using standard chemical spray application technology (Chapple *et al.* 2007). For fungal control agents, it is important to use the right propagule, formulated in an appropriate manner and applied at the correct dosage and time. Fungal conidia, such as *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) and *Metarhizium anisopliae* (Metsch.) Sorokin (Hypocreales: Clavicipitaceae), have hydrophobic cell walls that make it difficult to suspend these spores in water. Also, some mechanical sprayers can adversely affect the viability and virulence of the entomopathogenic fungi used. The viability of oil-formulated conidia of *M. anisopliae acridum* is reduced by 30% after passage through an exhaust nozzle sprayer due to the brief exposure of conidia to temperatures over 100°C (Griffiths & Bateman 1997).

Researchers have also investigated the use of autodissemination devices that attract insect pests into a focus of entomopathogens (fungi, baculoviruses or bacteria) from which the microbial control agents can be disseminated to other members of the pest population (Hunter-Fujita *et al.* 1998). The main advantage of autodissemination technology is that delivery of the microbial agent is targeted to the pest species with limited detrimental effects to other organisms or the environment. With the increased availability of pheromones and other lures for agricultural pests, this application technology provides an additional delivery strategy for microbial control agents.

An extension of the autodissemination approach for delivery of microbial control agents is the development of the novel application strategy "Pollinator Biocontrol

Vector Technology” (PBVT). It has been known for a long time of the ability of bees to carry microscope particles such as pollen. In addition, it has been well documented that many of these microscopic particles include plant pathogenic fungal spores and bacterial cells (Morse & Nowogrodski 1990). Specific examples are the spread of the fire blight pathogen, *Erwinia amylovora* (Burrill) Winslow (Enterobacteriales: Enterobacteriaceae), to apple and pear blossoms (Johnson *et al.* 1993) and mummy berry disease, *Monilinia vaccinii-corymbosi* (Reade) Honey (Helotiales: Sclerotiniaceae), to blueberry flowers (Dedej *et al.* 2004) by honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). This knowledge has led to the idea that bees can also be used as carriers of biological control agents and has resulted in the development of the PBVT approach (Kevan *et al.* 2008). The main research and developmental processes and components for PBVT are illustrated by the flow diagram in Fig. 1 and show how the various components are integrated. This paper will describe how the authors have developed the PBVT approach using the bumble bee pollinator/greenhouse pest system for greenhouse vegetable crops and are expanding it to include other insects as vectors of fungal control agents.

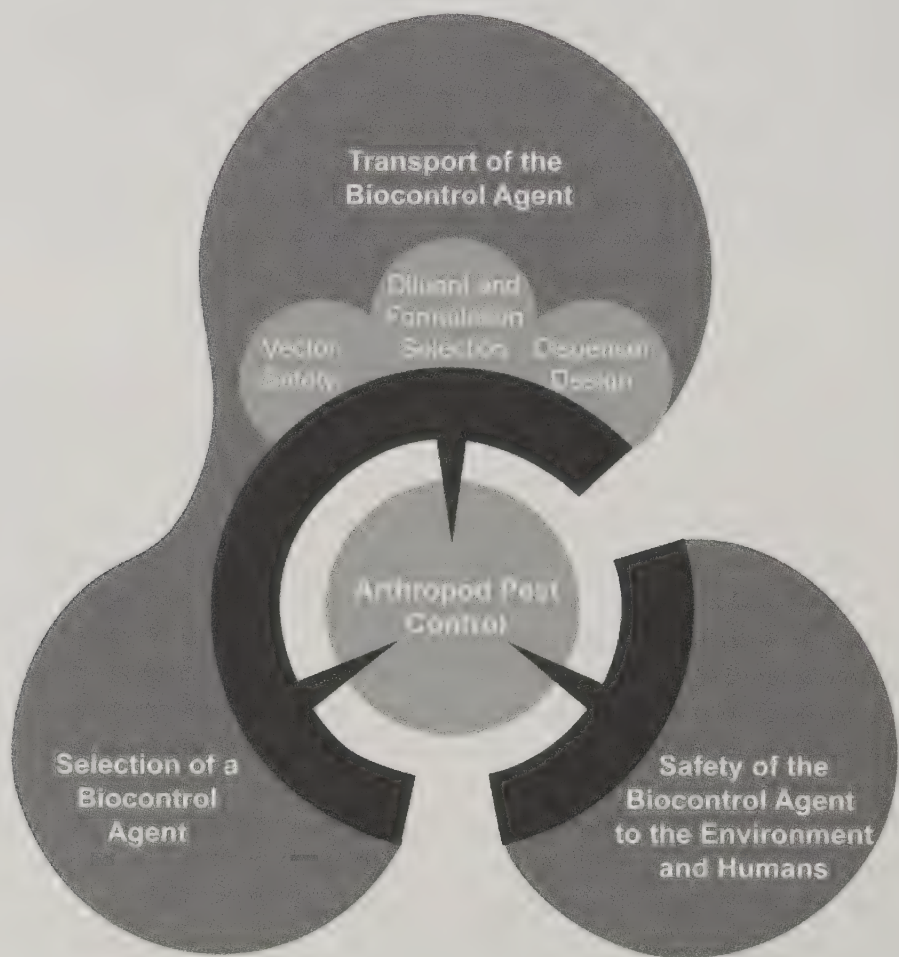


Fig. 1. A flow diagram of the different components of the Pollinator Biocontrol Vector Technology approach and how they are integrated.

SELECTION OF A BIOCONTROL AGENT.

The PBVT was first used to vector bacterial and fungal control agents for plant diseases for a range of horticultural crops (pome fruits, strawberries, raspberries and

blueberries) using honey bees and bumble bees (Dedej *et al.* 2004; Johnson *et al.* 1993; Kovach *et al.* 2000; Maccagnami *et al.* 1999; Peng *et al.* 1992; Yu & Sutton 1997). With arthropod pests, the initial work was conducted with *Heliothis* nuclear polyhedrosis virus (NPHV) for control of corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Tortricidae), on crimson clover using honey bees (Gross *et al.* 1994). Butt *et al.* (1998) demonstrated that honey bees could be used to vector the fungal control agent, *M. anisopliae*, for control of pollen beetle, *Meligethes aeneus* Fab. (Coleoptera: Nitidulidae), on rape. Next, Jyoti & Brewer (1999) showed that honey bees could deliver *Bacillus thuringiensis* (Berliner) (Bt) var. *kurstaki* (Bacillales: Bacillaceae) to sunflower flowers for control of banded sunflower moth, *Cochylis hospes* Walsingham (Lepidoptera: Tortricidae).

The first research to be conducted with bumble bees for arthropod pest control was with the fungal biocontrol agent, *B. bassiana* against tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), and western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), on greenhouse sweet pepper (Al-mazra'awi *et al.* 2006). The entomopathogenic fungus, *B. bassiana* was selected as candidate biocontrol agent for PBVT because it has been shown in previous studies to have good potential as a control agent for many agricultural pests including *L. lineolaris* and *F. occidentalis* (Bidochka *et al.* 1993; Gindin *et al.* 1996; Goettel *et al.* 1990). In addition, *B. bassiana* was registered as a microbial control for greenhouse pests in the US and has been submitted for registration against greenhouse pests to the Pest Management Regulatory Agency in Canada.

TRANSPORT OF THE BIOCONTROL AGENT.

Diluent and Formulation Selection.

For our work, we used *B. bassiana* in the commercial formulation (Botanigard 22WP[®], Laverlam International Corp., Butte, MT). However, the biocontrol agent was too concentrated in the commercial formulation and had to be diluted to maximize dispersibility and dosage at the target site and minimize impact on vector safety. Factors that affect the ability of a vector to acquire and deliver fungal spores include the type of carrier, particle size of the carrier, and moisture content. The type of diluent/carrier is very important when preparing the inoculum. Earlier studies found that some carriers can be irritants to the bees and honey bees spend a considerable amount of time grooming from their bodies (Israel & Boland 1993). To be an effective carrier, the diluent should be inexpensive, easily available and among the least irritating to the bees. We tested eight carrier types (corn flour, durum semolina, wheat flour, oat flour, potato starch, corn meal, graham cracker crumbs and potato flakes (Al-mazra'awi *et al.* 2007). Bees that passed through the corn flour acquired the most conidia of *B. bassiana* (1.7×10^6 colony forming units [cfu]/bee). The optimal particle size for the greatest acquisition of *Beauveria* spores (8.3×10^5 cfu/bee) was 45-90 μm . In addition, moisture content of the inoculum also affected the amount of *B. bassiana* acquired by the honey bees. More conidia (1.9×10^6 cfu/bee) were picked up from the drier inoculum (no evaporative water) compared to the inoculum that contained 50% (w/w) sterile water to dry corn flour. In all trials, time spent by the bees crawling through the inoculum had no effect on the amount of conidia that were acquired by the bees. The bodies of the bees became saturated with the inoculum in < 5 sec.

Dispenser Design.

Design of the inoculum tray is critical to the success of using insects to acquire sufficient amounts of the microbial control agent so it can infect its target host. Dispensers that direct the bees through the inoculum as they leave the hive and then isolate them from the inoculum when returning to the hive minimize wastage of the inoculum and help protect the colony in cases where the microbial control agent can also infect the vector. In our studies, we used an over-and-under design that forces the bees into a lower chamber containing the inoculum before exiting the dispenser and returning bees re-enter the hive by passing through the upper chamber of the dispenser that is inoculum free (Fig. 2). A side-by-side dispenser based on the same operating principles as the over-and-under design was developed by van Steen *et al.* (2006) for dissemination of *Ulocladium atrum* Preuss (Pleosporales: Pleosporaceae) by honey bees against grey mold in strawberries. Other dispenser types have been developed for honey bees that use a one-way dispenser (ie. the bees enter and exit through the inoculum tray (Bilu *et al.* 2004; Dedej *et al.* 2004; Gross *et al.* 1994; Thomson *et al.* 1992). However, these inoculum dispensers have only been used with honey bees. More research is needed with dispenser design to address inoculum acquisition by the vector, the viability of the inoculum over time, and dispenser interference with bee and colony activity through restriction of the hive entrance.



Fig. 2. The over-and-under inoculum dispenser used in the greenhouse bumble-bee vector trials with the lower tray extended to show the inoculum and zig-zag path that the bees follow to acquire the inoculum.

Vector Safety.

When selecting a biocontrol agent and inoculum formulation, it is essential that the inoculum is not harmful to the vector. The fungal biocontrol agent *Trichoderma harzianum* Rifai (Hypocreales: Hypocreaceae) which is used to suppress the incidence of grey mold has been evaluated for vector safety and found to be harmless to both honey bees and bumble bees (van der Steen *et al.* 2004). With

entomopathogenic control agents, Bt has been found to be safe with honey bees (Vandenburg & Shimanuki 1986). The NPHV used by Gross *et al.* (1994) for *H. zea* control is specific to Lepidoptera and thus, is expected to be safe to honey bees. With Botanigard 22WP, we found that the commercial concentration of *B. bassiana* had to be diluted from 2×10^{11} conidia/g of product to 6×10^{10} conidia/g for minimal mortality to the bumble bee *Bombus impatiens* (Cresson) (Hymenoptera: Apidae) and maximum infection of the pest species (Al-mazra'awi *et al.* 2006a&b; Kapongo *et al.* 2008a). Goettel & Jaronski (1997) reported that exposure of whole honey bee hives to *B. bassiana* GHA strain resulted in < 1% infection of the worker bees and no infection of the brood.

SUCCESSFUL CONTROL OF ARTHROPOD PESTS.

The PBVT approach has been evaluated successfully against insect pests of field and greenhouse crops. The initial research was conducted with honey bees delivering *Heliothis* NPHV for control of *H. zea*. Mortality levels reaching 73-87% were found in the treatment fields compared to 11-14% in the control fields (Gross *et al.* 1994). Next, honey bees were used to vector *M. anisopliae* for *M. aeneus* control with 61% mortality on winter rape and 100% mortality on spring rape (Butt *et al.* 1998). Carreck *et al.* (2007) later showed that bee-vectored *M. anisopliae* can cause infection and mortality of larval and adult *M. aeneus* and adult cabbage seed weevil, *Ceutorhynchus obstrictus* (Marsham) (= *C. assimilis* (Paykull) (Coleoptera: Curculionidae). In Canada, honey bees have been used to deliver *B. bassiana* for control of *L. lineolaris* on canola (Al-mazra'awi *et al.* 2006b). Lastly, Jyoti and Brewer (1999) demonstrated that honey bees can be effective vectors of the bacterium Bt var. *kurstaki* for control of *C. hospes*. The mortality achieved using the PBVT was equivalent or better than spray application of Bt.

With greenhouse crops, the initial proof of concept was conducted with the commercial bumble bee pollinator *B. impatiens* as a vector of *B. bassiana* for control of *L. lineolaris* and *F. occidentalis* (Al-mazra'awi *et al.* 2006a). *Lygus lineolaris* were a new pest of greenhouse crops and there was no biological control agent available that would provide effective control preventing economic damage to the crop. With an inoculum concentration of 1.0×10^9 cfu/g of inoculum, we showed that adult *L. lineolaris* mortality ranged from 34-45% for the bee-vectored treatment versus 9-15% for the non-bee vectored control. The infection rates for adult *F. occidentalis* ranged from 34-40% for the *B. bassiana* treatment compared to 3% for the control. Essentially all the bee samples and 87-96% of the flower and leaf samples contained detectable concentrations of *B. bassiana*. Thirty-fourty-two percent of the collected adult *L. lineolaris* contained 587-708 cfu/insect.

Based on these positive results, we expanded our research to include greenhouse tomato as well as sweet pepper and more pests (greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) and green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae)) in addition to *L. lineolaris* (Kapongo *et al.* 2008a). These trials determined the optimal concentration of *B. bassiana* that can be used in the inoculum to provide maximum infection of the pests and minimal impact on the pollinator. The optimal concentration of 6.24×10^{10} conidia/g of inoculum resulted in mortality levels of 54% for adult *T. vaporariorum* and 70% for adult *L. lineolaris*. Nymphal samples of *T. vaporariorum* and *M. persicae* were collected, but

individual mortalities were not determined because mortality from handling these insects was too great. Internal infection levels after surface sterilization of the nymphs were 34% for *M. persicae* and 29% for *T. vaporariorum* at the optimal *Beauveria* concentration. Leaf samples were collected at the top, middle and bottom of the plant canopy each sample date to determine the vertical profile for spore deposition from *B. impatiens* vectoring. The greatest numbers of spores were collected from the top one third of the canopy. This is not surprising as this is the section where the flowers are found. However, spores were detected throughout the complete canopy. Bumble bees are buzz pollinators whereby high frequency muscle vibrations are used to shake the pollen off flowers and bees were often seen grooming themselves on the leaves. These activities combined with air movement can result in the deposition of the inoculum throughout the plant canopy. Again, in the trials by Kapongo and colleagues, the percentage of bee, flower and leaf samples from both crops containing detectable concentrations of *B. bassiana* ranged from 75-100% for the optimal *Beauveria* concentration treatment.

SAFETY OF THE BIOCONTROL AGENT TO THE ENVIRONMENT AND HUMANS.

From an environmental and human safety perspective, some of the biocontrol agents that have been evaluated using the PBVT approach are already considered safe and have been registered with the appropriate agencies in various countries for application to crops. The advantage of the PBVT approach is that the inoculum is delivered directly to the flowers and leaves where the pests are found. There is continuous dissemination of the microbial control agent as the bees are pollinating the plants daily compared to a single point in time when the microbial agent is sprayed on the crop. As a result, there is less wastage of the material into the soil and air. Allergic reaction of *B. bassiana* can potentially be a human safety issue. However, Goettel and Jaronski (1997) report that no incidents of human hypersensitivity reactions were noticed by workers of the company Mycotech during the many years of mass production of this fungus. A recent review on the safety of *B. bassiana* found that this fungal control agent does not pose any serious detrimental effects to environmental and human health and should be considered safe (Zimmermann 2007).

DISCUSSION.

Compatibility with Other Integrated Pest Management Strategies and Crop Production Practices.

Combining pollination with the benefit of pest management is a win-win situation for growers, especially for crops where pollination is not used now or used only on a limited basis. For example, insect pollination of canola improves seed quality and germination rate (Kevan & Eisikowitch 1990) and results in higher seed set and yields (Langridge & Goodman 1975). In greenhouse sweet pepper, using bumble bees results in increased fruit weight, volume, seed weight and percentage of extra-large and large fruits, and reduced the number of days to harvest (Shipp *et al.* 1994). In greenhouse tomatoes where bumble bees are the industry norm for pollination, it is important to ensure that application of bee vectored microbes does not reduce the pollination efficiency of bumble bees to the crop and resulting fruit quality and yield. To evaluate this situation, bumble-bee vectored *B. bassiana* was assessed at three

commercial greenhouses in 2006 over a seven-week period (Kapongo 2007). The presence of the dispenser attached to the exit/entry portal of the hive reduced bee activity exiting and re-entering the hive. However, pollination levels at all three greenhouses were considered acceptable for good fruit production in commercial greenhouses. Also, 75% of the fruit yield weighed ≥ 200 g/fruit and contained a mean seed count of > 250 seeds/fruit which is an indicator of normal production of beefsteak tomato under commercial production conditions. In addition, recent greenhouse cage trials by the authors with parasitoids and predatory bugs and mites commonly used on greenhouse crops found that exposure to bee-vectored *B. bassiana* did not have any negative impact on their parasitism or predation rates. Mortality rates were similar between the control (no bee-vectored *Beauveria*) and the bee-vectored *Beauveria* treatments.

Future Directions.

In the earlier trials with *B. bassiana*, the fungal control agent was diluted with corn flour to reduce the concentration of the commercial product (Botanigard 22WP[®]) to the desired concentration for the bees to deliver it to the crop. Kapongo *et al.* (2008) replaced the diluent with another beneficial fungal agent, *Clonostachys rosea* (Link: Fries) Schroers, Samuels, Seifert, and Gams (Ascomycota: Hypocreales), to determine if two agents can be combined into a single inoculum for delivery by *B. impatiens* for arthropod pest control and plant disease suppression. When the bee-vectored inoculum was applied to tomato plants in greenhouse cage trials, 49% of the collected adult *T. vaporariorum* were killed and grey mould was suppressed by 57% and 46%, respectively, on the flowers and leaves. For sweet pepper, mortality for adult *Lygus* was 73% and grey mould was again suppressed by 59% and 47%, respectively on the flowers and leaves. Subsequent trials have investigated the optimal concentration for each agent when combined into a single inoculum. Thus, it is possible to use PBVT to simultaneously vector fungal agents for insect pest control and plant disease suppression. In fact, there may be still room in the inoculum to add a third agent. The ability of pollinators to deliver multiple control agents at the same time is exciting and important from a pest management perspective, but also raises the question of possible intra-guild interactions among microbial control agents. Using pollinators can thus potentially revolutionize how microbial control agents are applied to greenhouse crops.

We are also investigating the use of pest insects as vectors of beneficial fungal agents. It is well documented that adult and larval fungus gnats, *Bradysia impatiens* (Johannsen) (Diptera: Sciaridae) can transmit root and foliar plant pathogens such as *Botrytis*, *Fusarium* and *Pythium* by carrying spores on their bodies or via their feces (Gardiner *et al.* 1990; Jarvis *et al.* 1993). It was hypothesized that these pests can also carry beneficial fungi (ie. *C. rosea*) the same manner. Petri dish trials have found that adult and larval fungus gnats can vector externally enough spores of *C. rosea* to suppress *Pythium* growth on the plates. This research is just at the beginning stages, but illustrates that other insects including pests can potentially be used to vector microbial control agents. Using insects to deliver microbial control agents should be view as another pest management tool that needs to be integrated into an overall biological control program approach for arthropod pests.

CONCLUSIONS.

Pollinator Biocontrol Vector Technology is a multidisciplinary pest management approach that incorporates different ecosystem components such as pollinators, microbial control agents and insect pests in the crop production system. It brings the benefits of biological control, reduced chemical use, and better pollination of the crop, all of which result in higher yields and improved crop quality.

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INFLUENCE OF UV-ABSORBING NETS IN THE POPULATION OF *MACROSIPHUM EUPORBIAE* THOMAS (HOMOPTERA: APHIDIDAE) AND THE PARASITOID *APHIDIUS ERVI* (HALIDAY) (HYMENOPTERA: APHIDIIDAE) IN LETTUCE CROPS

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ABSTRACT.

A lettuce crop was grown under two tunnels (8m x 6.5m), one covered by a 50 mesh UV-absorbing net (BioNet[®]), and another by a 50 mesh standard net. Each tunnel was divided into four identical sealed compartments where 22 marked plants, two per row, were artificially infested, three days after transplanting, with three alate *Macrosiphum euphorbiae* per plant. One month later, 30 adult *Aphidius ervi* parasitoids were released into the centre of each compartment and further releases were made weekly for four weeks. The marked plants were monitored by visual inspection each week for up to 10 weeks and scale counts were made. The number of aphids under the standard net tunnel was significantly higher than under the UV-absorbing net (BioNet[®]) from the fourth sampling time while the number of parasitoid mummies counted was rather similar. The results suggest that UV-absorbing nets in combination with parasitoid releases could be an effective and compatible alternative to control aphids on lettuce crops in greenhouses.

INTRODUCTION.

Among the important factors limiting yields of lettuce, *Lactuca sativa* L. (Asteraceae) in both outdoor or greenhouses environments in many regions of Spain, is the lettuce mosaic virus (LMV) which is transmitted by aphids such as *Macrosiphum euphorbiae* Thomas (Homoptera: Aphididae) (Jordá 1991). The transmission time for the virus is just few seconds, therefore chemical control of the vector species is largely ineffective because pesticides require more time to take effect (Perring *et al.* 1999). Thus, it continues to be important to look for alternative vector control measures and globally, environmentally friendly tactics such as photoselective barriers and biological control are being sought.

UV-absorbing nets can prevent insects from entering greenhouses, will affect their flight behaviour, and their behaviour for selecting and assessing the quality of host plants through interference with their visual orientation and movement (Díaz *et al.* 2006, Weintraub & Berlinger 2004). The consequence of this interference is to reduce the dispersion of the virus.

Biological control is a key tool in sustainable agriculture (Viñuela 2005) and the importance of preserving or enhancing natural enemies in agroecosystems is stressed in Spanish and European Union (EU) directives that deal with pesticide use or pest control in modern production systems. The EU directive 91/414/CEE and its

modifications (DOCE 1991, 1996) was a global pioneer in establishing mandatory ecotoxicological studies on beneficial fauna prior the registration of pesticides. Furthermore, the EU Directive for Organic Production highlights the great importance of natural enemies in the control of pests (DOCE 2002). Similarly, in Spain, the protection of the associated fauna is considered of key importance in horticultural crops under Integrated Production (17,500 ha in 2006) (Boe 2004a). The National program, started in 2004, aims to control insect vectors of viruses in organic crops and encourages the adoption of biological control strategies through support to farmers (Boe 2004b).

UV-absorbent films are effective in the control of pests such as aphids and, while they extend the persistence and feasibility of entomopathogens (Costa *et al.* 2001; Fereres *et al.* 2003), they do not appear to affect the behaviour of parasitoids (Díaz & Fereres 2007). The experiments described here aimed to improve understanding of the effects of a UV-absorbent net on the population growth of the pest *M. euphorbiae* and its parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Aphidiidae) in a lettuce crop.

MATERIALS AND METHODS.

The experiments were carried out under field conditions in spring 2008 at the “La Poveda” experimental farm in Madrid, central Spain. Two tunnel type greenhouses (6.5 x 8 m) were covered by 50 mesh size nets: one standard (Criado & López, Spain) and the other photoselective (Bionet[®]; Tripiñana S.L., Spain) which absorbs UV-radiation in the UV-A and UV-B range from 400 to 280 nm. Each tunnel had four sealed compartments with individual entrances. In each compartment, 66 variety Aitana lettuce plants, (BBCH stage 13; 3 leaves; Meier 2001) were planted in 11 rows and provided with drip irrigation. One week later, three alate *M. euphorbiae* aphids were introduced to every third plant, which were marked for further monitoring (22 plants in every compartment).

Temperature and relative humidity inside the greenhouses were continuously monitored with Tinytag[®] data loggers (Gemini, UK), and rainfall and outdoor temperature were also recorded. Photosynthetically active (PAR) and ultraviolet (UV) radiation inside and outside the greenhouses were recorded weekly at 12h00 with Quantum meter radiometers (Apogee, USA), models BQM and UVM, respectively.

Direct visual sampling was conducted during a 10 week period on the marked plants. The number of aphids (alate and apterous) was recorded based on scale counts: 0 = 0 aphids, 1 = 1-4 aphids, 2 = 5-19 aphids, 3 = 20-49 aphids, 4 = 50-149 aphids, 5 > 150 aphids. BBCH crop stage was recorded as well (Meier 2001).

When the density of aphids was high enough (i.e., when scale three (20-49 aphids/plant) was predominant, four weeks after transplant), the first parasitoid release was made by placing 30 mature *A. ervi* mummies on a small cap, placed directly on the ground in the middle of each compartment (approximately 2 insects/m², the highest or four times the highest recommended commercial rate by Biobest (2008) and Koppert (2008) Spain, respectively). Further releases were done during the next four weeks. The number of mummies on the marked plants was recorded on a weekly basis.

At harvest time (day 49 after transplant; BBCH stage 49, typical leaf mass), a final destructive sampling was conducted by cutting eight lettuce plants at the base (2 per compartment) in each greenhouse type and placing the plants in labelled polythene bags. In the laboratory, actual numbers of aphids and parasitoids were recorded using a binocular stereomicroscope. The weight of every marked plant was recorded as well.

Aphid scale counts in samplings were transformed into number of aphids for analysis as follows: (0 = 0 aphids, 1 = 3 aphids, 2 = 12 aphids, 3 = 34 aphids, 4 = 100 aphids; 5 = 150 aphids). To compare the two types of netting (treatments), a one-way analysis of variance (ANOVA) was performed for each sampling date on aphid and parasitoid counts, on PAR and UV radiation inside the netting, and on crop production, after variance and normality check analysis using Statgraphics® version 5.1 (Stsc 1987). Means were separated by the LSD multiple range test ($P < 0.05$). If any of the assumptions of the analysis were not met even after transformation to $\log(x+1)$, a non-parametric Kruskal-Wallis test was applied and medians were used to compare the different groups (Zar 1996). Data presented in the tables are means \pm SE.

RESULTS AND DISCUSSION.

The average numbers of *M. euphorbiae* aphids (alate and apterous) and parasitoid mummies every sampling date in the lettuce plants under control and UV-absorbing nets are shown in Table 1. The population dynamics of *M. euphorbiae* based on visual scale counts are shown in Fig. 1.

Table 1. Mean number (\pm se) of aphids, *Macrosiphum euphorbiae*, and *Aphidius ervi* parasitoid mummies on lettuce plants (n=88) under the two types of tunnels, based on visual scale counts*.

Sample date	<i>M. euphorbiae</i> /plant		<i>A. ervi</i> mummies/plant**	
	Control	UV-absorbent	Control	UV-absorbent
11 March 08	5.87 \pm 0.57a	4.16 \pm 0.36a	-	-
14 March 08	9.90 \pm 0.73a	8.78 \pm 0.64a	-	-
18 March 08	14.77 \pm 1.02a	13.03 \pm 0.89a	-	-
28 March 08	30.65 \pm 3.02a	19.19 \pm 1.54b	-	-
2 April 08	47.50 \pm 3.77a	22.02 \pm 1.75b	-	-
9 April 08	70.14 \pm 3.96a	52.57 \pm 1.75b	0.03 \pm 0.02a	0.03 \pm 0.03a
16 April 08	77.25 \pm 4.57a	55.14 \pm 3.87b	0.39 \pm 0.08a	0.03 \pm 0.02b
23 April 08	100.31 \pm 4.81a	73.86 \pm 4.48b	8.25 \pm 0.63a	6.31 \pm 0.49b
30 April 08	119.36 \pm 4.79a	78.23 \pm 5.53b	16.51 \pm 1.40a	15.54 \pm 1.20a
7 May 08	150.00 \pm 0.00a	150.00 \pm 0.00a	37.88 \pm 7.74a	25.25 \pm 2.46a

*Visual scale counts transformed into number of aphids as follows: 0 = 0 aphids, 1 = 3 aphids, 2 = 12 aphids, 3 = 34 aphids, 4 = 100 aphids; 5 = 150 aphids. Within line and factor, values followed by the same letter are statistically equal (ANOVA and LSD; ^{1,9,6**},^{7**},^{10**} Kruskal-Wallis; $P < 0.05$).

Nakagaki *et al.* (1982) was the first to demonstrate that UV-absorbing nets may reduce insect invasion in greenhouses. This is corroborated by results of this study in that *M. euphorbiae* densities were always lower in the greenhouse covered by the UV-absorbent Bionet®. This suggests that this UV-blocking material interferes with the vision of aphids and, in consequence, with their behaviour related to movement, host location and population dynamics. Similar results have been reported for other pest species (see review of Díaz & Fereres 2007). Actual aphid numbers in the crop assessed by the final destructive sampling were very high: 1500.0 ±370.0 and 1184.3 ±143.5 aphids in the control and UV-absorbent treatments, respectively.

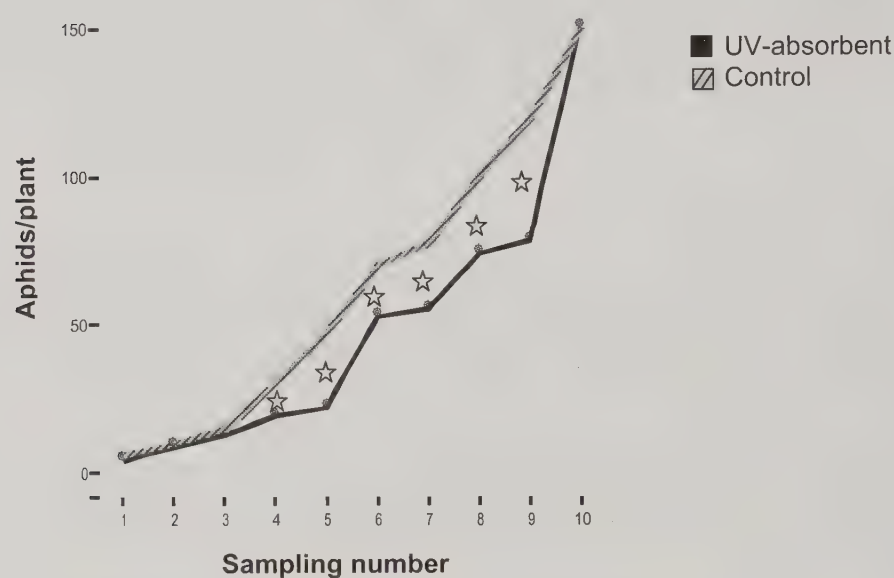


Fig. 1. Population dynamics of *Macrosiphum euphorbiae* in the lettuce crop under control and UV-absorbent nets. Mean numbers of aphids based on visual scale counts in the field transformed as follows: 0 = 0 aphids, 1 = 3 aphids, 2 = 12 aphids, 3 = 34 aphids, 4 = 100 aphids; 5 = 150 aphids. Stars indicate significant differences between the means (ANOVA, LSD, $P < 0.05$).

The percentage of each aphid scale count at every sample date in control and UV-absorbent treatments as well as their duration are shown in Fig. 2. The lowest count, 0, was recorded on some marked plants up to the fifth sample time, scale counts two and three were dominant in most of the samples, and one week before harvesting (sample nine) the highest scale count, 5, was detected in less than 30% of the plants. This indicates that it took longer for the aphids to establish significant numbers in the lettuce crop under the UV-absorbent net. In contrast, in the control, aphids were present with different intensity in every marked plant from the third sample time onwards, higher scale counts (2-4) were more prevalent and one week

before harvest, the highest scale count, 5, was recorded in more than 60% of the plants.

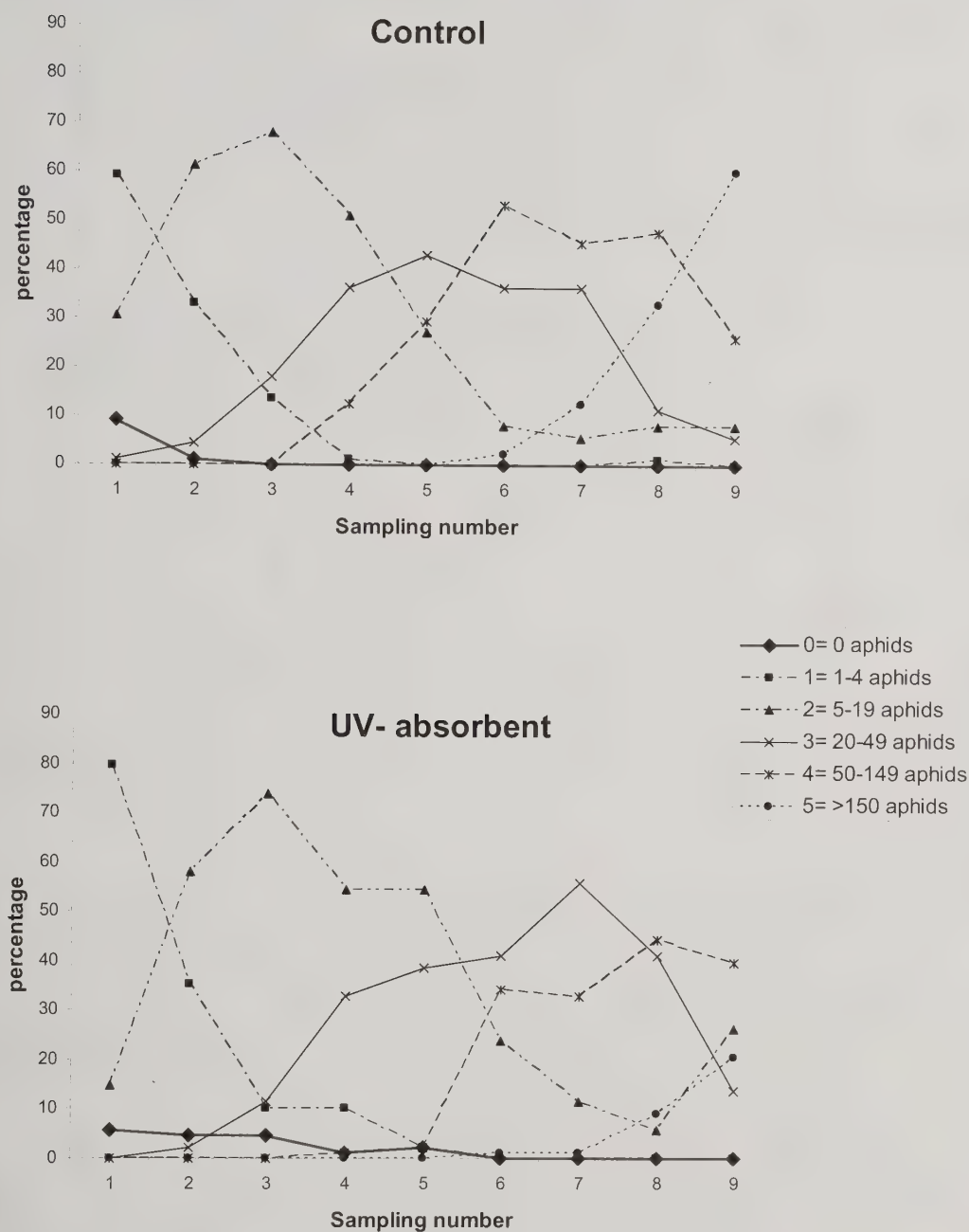


Fig. 2. Frequency (percentage) on each sample date of each scale count of aphids on lettuce plants in control and UV-absorbent treatments.

Climatic conditions recorded during the experiment are presented in Table 2. Inside the UV-absorbent treatment, maximum temperature was lower than under the control treatment while the average RH was higher. Under the UV-absorbent treatment, the percent reduction in PAR radiation was slightly higher and statistically significant differences were detected in the percentage reduction of UV radiation

(47.325 and 79.55 micromol m⁻²s⁻² under the control and the UV-absorbent treatments, respectively). During the crop cycle, total rainfall was not high (58.2 mm) and it was mainly concentrated during 2 weeks (27.2 mm between sample times 3-10 April and 22.4 mm between 17-23 April).

Table 2. Climatic conditions in the lettuce crop (March-May 2008; La Poveda, Central Spain).

			Outside tunnels			
Rainfall (mm)	58.2					
	max	min	average			
Temperature (°C)	28.9	-5.8	12.03			
			Inside tunnels			
			Control		UV-absorbent	
	max	min	average	max	min	average
Temperature (°C)	42.4	-3.51	15.73	37.83	-3.04	14.98
RH (%)	100	9.9	58.44	100	8.7	60.19
PAR radiation reduction (%)	49			63		
UV radiation reduction (%)	47.32a			79.55b		

In contrast to the aphid counts, the number of parasitoid mummies was practically identical in both tunnels, except at harvest (sample 10), when the numbers were higher in the control treatment (Fig. 3). The average number of *A. ervi* mummies/plant never surpassed 15, except at harvest, indicating that the parasitoid reproduction rate was not very high under our experimental conditions, irrespective of the type of net.

At harvest, the average weight of lettuces under the control net was significantly higher than under the UV-absorbent net (622 ±23.41 g and 431 ±23.52 g, respectively; ANOVA, LSD, *P* < 0.05), but all lettuces were marketable because weights were always higher than 100g (DOCE, 2001). However, none of the lettuces under the control or under the UV-absorbent treatments were marketable based on aphid infestation, because the number of aphids/lettuce was very high (prevalence of scale count 5). Accordingly, the population density of *A. ervi* was not high enough in either treatment to control the aphids, even though the release rates (2 parasitoids/m²) were higher than those recommended for commercial greenhouses. The heavy rainfall during the two weeks in April (22. 4 mm) could have accounted for such a result, because many parasitoid adults were probably killed before being able to parasitize the aphids.

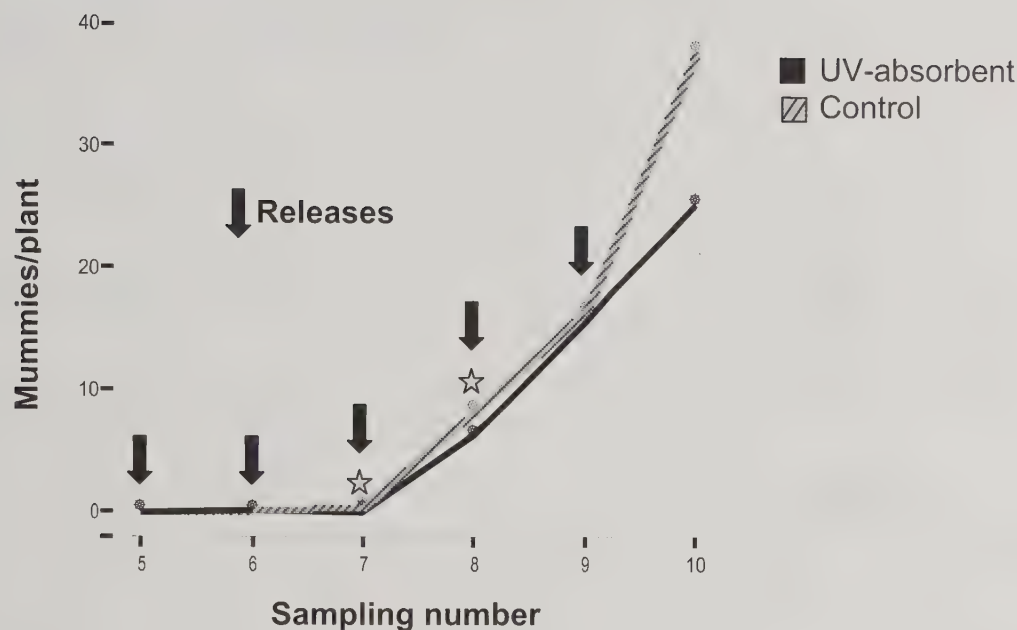


Fig. 3. Mean number (\pm se) of *Aphidius ervi* mummies in the lettuce crop under control and UV-absorbent treatments. Sample times 1 to 9 based on visual counts in the field; sample time 10 based on a visual count under the stereomicroscope in the laboratory. Stars indicate significant differences (ANOVA, LSD, $P < 0.05$).

CONCLUSIONS.

The Bionet[®] net reduced the total number of *M. euphorbiae* attacking lettuce plants and the percent of plants infested with the highest scale counts was also reduced. The occurrence of the highest scale counts also appeared later on the season. In contrast, the average number of *A. ervi* mummies/plant was practically equal under the two types of nets suggesting that UV-absorbing nets in combination with parasitoid releases could be an effective and compatible alternative to reduce *M. euphorbiae* damage in greenhouse lettuce crops.

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SESSION 11

BIOLOGICAL CONTROL OF PHYTOPHAGOUS MITES - THEORY AND PRACTICE

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Phytophagous mites are small creatures and can go unnoticed when population levels are low. However, given the right conditions populations escalate rapidly often causing foliar and in some cases direct damage to fruit. In protected crops developing release strategies and optimizing timing can improve the efficacy of commercial acarine biocontrol agents (ABAs). While in more stable environments such as orchard crops, identification of indigenous ABAs and promotion of these predators coupled with the development of conservation techniques seem to be necessary for sustainable biological control. In this session authors will present studies utilizing innovative approaches for the control of phytophagous mites. Our aim is to expose the audience to the limitations of phytophagous mite control with ABAs and to explain how improved protocols for application and conservation management can improve the efficacy of these predators. These pertain both to ABAs specializing in mite control of *Tetranychus* species and to generalist predators feeding on an array of phytophagous mites. Finally, ground cover management as a means to promote ABA populations and reduce pest damage in different tree cropping systems will also be addressed. It is our hope that participants will gain more of an understanding of how ABAs can be used effectively for the control of phytophagous mites.

POLLEN PROVISIONING ENHANCES *EUSEIUS SCUTALIS* (PHYTOSEIIDAE) POPULATIONS AND IMPROVES CONTROL OF *OLIGONYCHUS PERSEAE* (TETRANYCHIDAE)

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ABSTRACT.

The perseae mite, *Oligonychus perseae*, a spider mite originating in Central America, is an important pest of avocado in California, Spain and Israel. Since *Euseius scutalis* is the most dominant phytoseiid predator in Israeli avocado orchards, and because all *Euseius* species are known to be specialized pollen feeders, we set out to determine whether pollen provisioning could: 1) enhance populations of *E. scutalis* and 2) improve perseae mite control. Toward these goals, we conducted experiments at two spatial scales: on seedlings in a climate chamber and in the orchard. Pollen was provisioned manually in the seedling trial, with a prototype pollen applicator in the first field trial, and it was windborne from patches of Rhodes grass (*Chloris gayana*) in the second field trial. In the seedling experiment, *E. scutalis* left pollen-free plants and, as a result, *O. perseae* populations persisted. On the pollen-provisioned plants in contrast, *E. scutalis* populations increased to high levels and *O. perseae* populations dropped to zero. Similar results were recorded in the first field trial. High predator and low pest populations inhabited trees that received pollen applications. Number of female predators on trees adjacent to the Rhodes grass patches in the second field experiment were almost an order of magnitude higher than those on trees five rows away. The importance of pollen as a food supplement for the conservation of indigenous phytoseiid predators and methodologies for pollen application are discussed.

INTRODUCTION.

The perseae mite, *Oligonychus perseae* Tuttle, Baker and Abbatiello (Acari: Tetranychidae), a spider mite originating in Central America, is a pest of avocado, inflicting economic damage in California (Kerguelen & Hoddle 1999a), Spain (González-Fernández *et al.* 2008) and Israel (Maoz *et al.* 2007). The mite colonizes the bottom of the leaves, spinning densely woven nests along the leaf veins causing substantial foliar damage to the 'Hass' avocado cultivar (Aponte & McMurtry, 1997). *Oligonychus perseae* was first discovered in Israel in the autumn of 2001 in several avocado plots in the Western Galilee. Since then, it has spread to all avocado growing areas (from North to South) in the Upper Galilee, Jezreel Valley, Efraim Hills, Carmel Coastal Plain, Hefer Valley and Rehovot-Lachish, causing extensive foliar damage and leaf drop in most of these regions. During field monitoring for perseae

mite we often observed *Euseius scutalis* (Athias-Henriot) (Acari: Phytoseiidae) feeding on *O. perseae* (Maoz *et al.* 2007).

In California, populations of a similar species, *Amblyseius* (= *Euseius*) *hibisci* (Chant), increased in spring in the presence of avocado pollen even in the absence of mite or insect prey (McMurtry & Johnson 1965). McMurtry & Scriven (1966) conducted greenhouse trials on avocado seedlings to determine the effect of pollen addition on fecundity of the predatory mite and its predation of *Oligonychus punicae* (Hirst), another spider mite pest of avocado. While the absolute number of spider mites consumed per predator was low in the presence of pollen, its fecundity was significantly higher and immatures developed faster than on prey diet alone. The authors concluded that the increase in reproduction and population size of the predator would more than make up for any reduction in absolute number of prey consumed per predator, thereby causing better overall pest control. Thus, despite the relatively low fecundity on spider mites and apparent inability of this predator to penetrate the web of the prey, McMurtry (1985) indicated that *E. hibisci* may be responsible for suppressing *O. punicae* to low levels. In summer however, when pollen is not available, predator populations often remain low and thus are unable to respond and prevent the increase of pest populations.

Following the introduction of *O. perseae* to California in the early 1990's specialized spider mite predators were sought out and evaluated (Hoddle *et al.* 1999; Hoddle *et al.* 2000; Kerguelen & Hoddle 1999b). Two phytoseiid predators, *Neoseiulus californicus* (McGregor) and *Galendromus Helveolus* (Chant), were identified and release rates were determined. Unfortunately, growers did not incorporate the releases of these acarine biological control agents into their IPM scheme because the cost of these inundative releases (2000 mites/tree) was more than ten times that of an aerial application of NR 415 oil (Hoddle *et al.* 2000). It is also interesting to note that augmentative releases of *N. californicus*, an indigenous predatory mite of California, have not lead to the establishment of the predator in California avocado orchards. Thus, annual releases of *N. californicus* are needed to attain perseae mite control.

Euseius stipulatus Athias-Henriot and *N. californicus* are the two indigenous phytoseiid predators associated with the perseae mite in avocado orchards of South-eastern Spain. The pest was first recorded in 2004 in the provinces of Málaga and Granada (González-Fernández *et al.* 2008). Recently, these researchers demonstrated that wind borne pollen, released from corn plants planted between the avocado rows, enhanced the populations of these two predators and improved perseae mite control. While this methodology served to test their working hypothesis, it cannot be considered for commercial application for numerous reasons, but mainly because pollen shedding by corn plants lasts for only one week. Accordingly, the authors suggested that corn pollen could be harvested and applied with an appropriate applicator when needed.

A more sustainable approach for pollen provisioning for enhancing populations of generalist phytoseiid predators could be the use of cover crops, commonly used by growers for soil conservation and weed management. Smith & Papacek (1991) demonstrated the importance of Rhodes grass *Chloris gayana* for the conservation of

Amblyseius (= *Euseius*) *victoriensis* (Womersley) thereby improving the control of two eriophyoid species in Australian citrus.

To identify the indigenous predatory mite fauna on avocado in Israel, a survey was conducted in 2002 and 2003. *Euseius scutalis* was by far the most abundant species, accounting for more than 96% of the phytoseiid fauna found on the leaves. To improve the biological control of perseas in Israel, we imported the specialized predator *N. californicus* and evaluated the efficacy of inundative releases in 2004 and 2005 (Maoz *et al.* 2007). Despite a significant reduction of 30% in seasonal cumulative mite days (CMDs) following *N. californicus* releases, leaf damage was still considerable and similar to control trees. Furthermore, phytoseiid predators recovered from all release plots were mostly of the species *E. scutalis* (78-95%). In parallel to our experiments with exotic phytoseiids, we began evaluating the potential of the indigenous *E. scutalis* for perseas in the lab with leaf disc experiments and in the field by monitoring pest predator populations in an organic orchard, where no control practices for perseas were performed. In the lab we obtained a significant reduction of perseas in our leaf disc experiments but our seasonal field samplings, conducted in late summer and autumn, suggested that low populations of this predator occur despite the presence of the pest in high densities (Maoz *et al.* 2007). This shortcoming led us to focus on evaluating the importance of alternative foods for the conservation of this predator. In the present study our objectives were to determine whether pollen provisioning could: 1) enhance populations of *E. scutalis* and 2) improve perseas control. To test these hypotheses we conducted experiments at two spatial scales; on seedlings in a climate chamber and in the orchard.

MATERIALS AND METHODS.

For the seedling trial (conducted at $25^{\circ}\text{C} \pm 1$, RH $56 \pm 2\%$, 14:10 h L:D), both pest and predatory mites were collected from the field. Mite densities on pollen-provisioned vs. untreated control seedlings (cv. 'Haas') were compared in 24 replications. The experiment began by placing 10 predators per seedling and sprinkling a small amount of pollen (one shake of a salt shaker) twice a week, on one expanded leaf (located on the upper part of the plant) of each of all 48 seedlings. Once *E. scutalis* populations had established (after a fortnight), the pollen was removed from the control plants and all seedlings were infested with perseas (30 active stages per plant). For the remainder of the experiment (6 weeks), we continued to manually add pollen twice a week to the pollen-provisioned seedlings. Fortnightly, densities of mite populations (all life stages of the pest and all motile stages of the predatory mites) were recorded on one leaf per plant taken from the middle of the seedling.

Effect of pollen provisioning on predator and prey populations was assessed in July-October 2007 in an organic orchard. In this experiment, each tree served as a replicate and treatments were replicated five times. All trees were first sprayed with a fine mist of water (Gan-Mor *et al.* 2003). Then and every other week, pollen was applied onto the pollen-provisioned trees using a prototype of an electrostatic pollen applicator (Fig. 1). Pest mites (all motile stages) were counted in situ with an 8X lens using the fast field counting method developed by Machlitt (1998). Phytoseiids

(gravid females) were monitored by beating three strokes per limb (six limbs/site) and collecting the mites from the tray (Argov *et al.* 2002).

A second field experiment was performed to determine the effect of Rhodes grass, as a source of wind borne pollen, on predator and pest populations. Rhodes grass is a sub-humid and sub-tropical perennial grass known to have good drought and high salt tolerances. Patches of Rhodes grass (40 meters long and 2 meters wide) were established between the rows of 5-year-old Haas avocado trees by mid July 2008. Plants began to flower in mid-August, subsequently pest and predator populations were monitored as described for the first field experiment. Mite populations in avocado rows adjacent to Rhodes grass patches were compared to those five rows away.

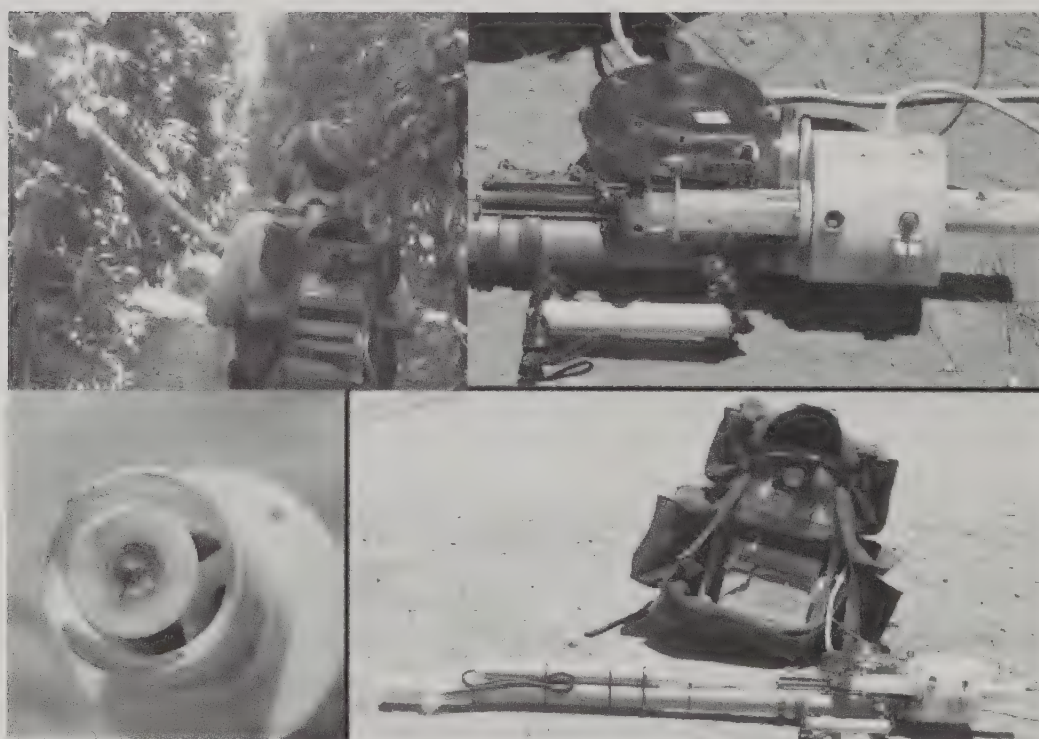


Fig. 1. Prototype electrostatic unit for pollen application (Patent Application No. US61/129,586).

RESULTS.

Significant differences in mite densities between pollen-provided and control treatments were recorded in the seedling experiment. Once pollen was discontinued in the non-treated control (two weeks into the experiment), *E. scutalis* left the plants and *O. perseae* populations persisted. On the pollen provisioned plants in contrast, *E. scutalis* populations increased to high levels of more than 30 mites per leaf whereas *O. perseae* populations dropped to zero within a week after infestation (Fig. 3).



Fig. 2. Flowering Rhodes grass *Chloris gayana* between rows of young avocado trees as a source of windborne pollen.

Similar results were recorded in the first field trial; higher predator and lower pest populations were found on the trees that received pollen applications. These differences, however, were not statistically significant (results are not presented).

In the second field experiment, density of female predators, pooled over six weekly counts, were almost an order of magnitude higher on trees adjacent to the Rhodes grass patches (179 female/tree) compared to those on trees five rows away (20 female/tree). These differences were highly significant ($t_{1,6}=10.148$; $p<0.0001$).

Surprisingly, no pest mites were found on leaf samples throughout the experiment in the studied orchard, or in any of the surrounding orchards. We suspect that the collapse of pest populations might be due to an unusually cold spell (several consecutive days with below freezing nightly temperatures) in the preceding winter.

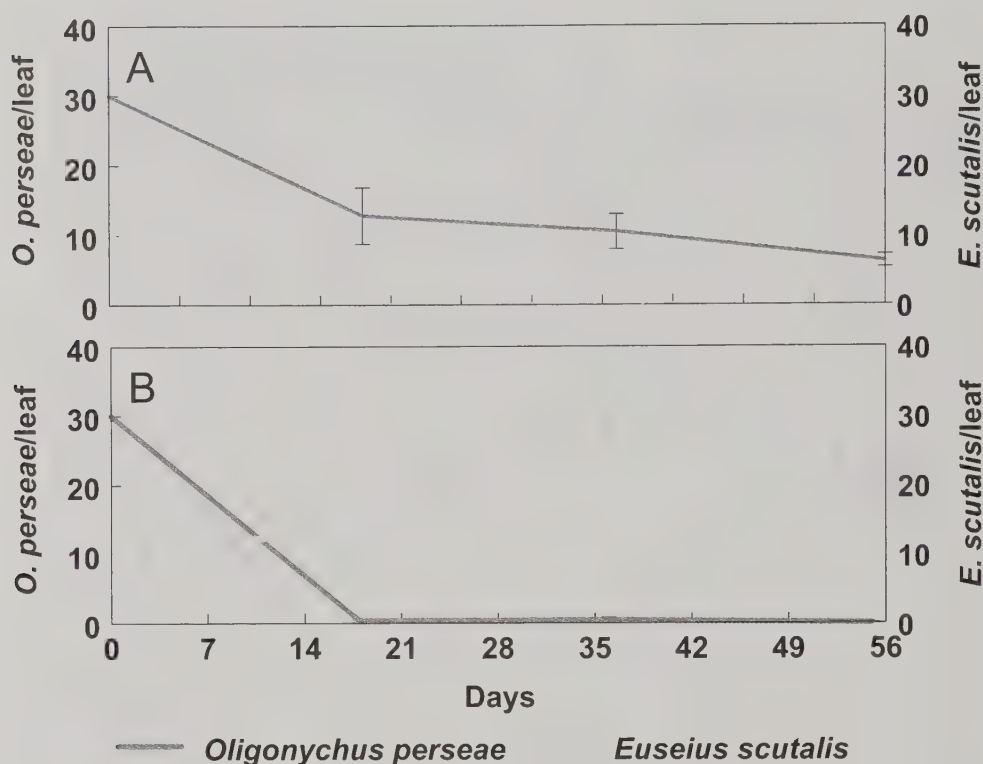


Fig. 3. Mean *Oligonychus perseae* and *Euseius scutalis* per leaf on avocado seedlings: A) Non treated control. B) With pollen provision.

DISCUSSION.

Euseius species are generalist predators commonly found in avocado orchards in California, south-eastern Spain and Israel. In the spring and early summer, when avocado pollen and other plant pollens are abundant, *Euseius* populations are usually high and *Oligonychus* populations are low (González-Fernández *et al.* 2008; Maoz *et al.* 2007; McMurtry 1985). Later in summer and into fall, however, predator populations usually decline despite the presence of high pest densities. To improve the biological control of the pest, efforts have focused on two different approaches: 1) Augmentation of introduced and indigenous specialized spider mite predators by conducting inundative releases of commercially, mass-reared phytoseiids.; and 2) Conservation of indigenous generalist predators. Because the first approach was found to be unsuccessful in Israel and non-sustainable in California, we focused on the second approach attempting to develop methods to enhance the activity of *E. scutalis*. Our seedling trial demonstrates that this predator can be a worthy candidate providing that an alternate food source is available. Comparable results were reported by McMurtry and Scriven (1966) in their avocado seedling studies with *E. hibisci* against *O. punicae*. More recent studies have demonstrated the importance of pollen as a food supplement for improving the establishment of *E. scutalis* for whitefly control on greenhouse cucumbers (Nomikou *et al.* 2003).

Pollen provisioning becomes considerably more challenging when moving from a protected (vegetables) or semi-protected environment (seedlings) to an open

orchard. When comparing the two techniques we tested for pollen provisioning, results suggest that windborne pollen released from Rhodes grass was far more effective in enhancing *E. scutalis* populations than fortnight pollen dusting with our electrostatic applicator. Rhodes grass flowers continuously and releases copious amounts of pollen throughout the summer months. This provides a constant flow of fresh windborne pollen to avocado foliage. Rhodes grass is also a robust plant suitable for soil conservation and tolerates trampling by farm machinery. On the other hand, Rhodes grass may compete with crop plants for water. This and other effects, such as that on weed management and exacerbation of frost damage, must await however further investigations. The final adoption of pollen delivery systems, be it through cover cropping or mechanical application, will depend of course on the overall economic and environmental benefit and cost analyses. Nonetheless, results of the present study and the cited literature strongly suggest that pollen is a suitable food supplement for establishing and conserving *Euseius* species in avocado orchards. Additional work is needed to refine conservation methods and to demonstrate the ability of *Euseius* species to prevent perseá mite outbreaks.

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RELEASE OF *NEOSEIULUS CALIFORNICUS* ON PEPPER TRANSPLANTS TO PROTECT GREENHOUSE-GROWN CROPS FROM EARLY BROAD MITE (*POLYPHAGOTARSONEMUS LATUS*) INFESTATIONS

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ABSTRACT.

Broad mites (*Polyphagotarsonemus latus*) rapidly damage pepper (*Capsicum annuum*) crops and reduce production in greenhouses in warm climate regions when the minute pest infests at early plant developmental stages. A first study was set to evaluate biological control strategies during seedling development. Timing of release of *Neoseiulus californicus*, a commercially-reared predatory mite, was investigated in scenarios of pest and predator presence to simulate preventive and curative control strategies. Undamaged seedlings were produced when two predators per seedling were released preventively. Curative control strategies were not effective for producing undamaged or broad mite-free transplants. Further studies were carried to evaluate the effectiveness of *N. californicus* on pepper seedlings that host a few broad mites and are transplanted into the production greenhouse. *N. californicus* released at different times and densities, and conventional control methods (micronized-sulfur sprays) were evaluated for pest control, plant damage, and fruit yield. Yields of red fruits from plants with four predators per plant released at any of the release times (six days before, at, or four days after transplanting) or with sulfur sprays were not significantly different from an uninfested control treatment. Releases of two predatory mites per plant led to yields similar to the uninfested control only when introductions were a week before transplanting, while later introductions led to serious plant damage and significantly lower yields. Early broad mite infestations in pepper crops grown in greenhouses might be effectively controlled with *N. californicus* released at early seedling developmental stages in the nursery and at transplanting in the fruit production greenhouse.

INTRODUCTION.

Infestations of broad mite [*Polyphagotarsonemus latus* (Banks) (Acari:Tarsonemidae)] initiated at early developmental stages of pepper (*Capsicum annuum*) can lead to severe plant damage in greenhouses where transplants or fruit are produced (de Coss-Romero & Peña, 1998; Gerson, 1992; Weintraub *et al.*,

2003). Seedlings will appear undamaged when they become infested with the minuscule mite a few days before transplanting but they carry the pest to crops grown in greenhouses. Biological control programs initiated at early pepper crop stages are disrupted by multiple sprays of commonly used pesticides (e.g., sulfur, abamectin, and dicofol). *Neoseiulus californicus* McGregor (Acari: Phytoseiidae), a predaceous mite that can be released on greenhouse-grown vegetables for biological control of two-spotted spider mite [*Tetranychus urticae* Koch (Acari: Tetranychidae)], has been reported to feed on *P. latus* and reproduce when feeding on this pest as well (Castagnoli & Falchini, 1993; Peña & Osborne, 1996). Preventive releases of *N. californicus* may provide effective pest management as the predator can remain on plants for short periods when prey is absent (de Courcy Williams *et al.*, 2004). Assessments of the effectiveness of releasing *N. californicus* on seedlings or young pepper plants have not evaluated the effects of the pest on fruit production in crops grown in greenhouses. This manuscript presents selected results of research in which we evaluated the use of *N. californicus* as a broad mite management strategy for greenhouse-grown pepper crops when infestations were initiated during seedling development.

MATERIALS AND METHODS.

In a first experiment, seedlings of bell pepper cv. 'Legionnaire' (Rogers-Syngenta) were grown in commercial nursery flats separated from each other by water barriers, and in a controlled environment (L:16h and D: 8h, with air temperatures $24 \pm 1^\circ\text{C}$ and $19 \pm 1^\circ\text{C}$, and air relative humidity $70 \pm 10\%$ and $80 \pm 10\%$, respectively, and no air movement over the seedlings). Seedlings were artificially infested with two gravid female *P. latus* at three seedling developmental stages (unfolded cotyledons, two leaves, and four leaves). Two *N. californicus* (reared by Biotactics, Romoland, California) were released on seedlings with a) unfolded cotyledons at either 0, +15, or +24 days from initial infestation (DFI) of *P. latus*, b) two leaves unfolded at either -15, 0, or +9 DFI, and c) four leaves unfolded, at either -24, -9, or 0 DFI. Additionally, separate groups of seedlings remained mite-free (non-infested control), and other groups were either infested with *P. latus* only or had releases of *N. californicus* only at the three seedling developmental stages. From each flat, seedlings were sampled every third day until day 42 after seeding. Mites were recovered using a plant washing procedure and counted under the microscope (Jovicich, 2007). Plant growth variables (leaf area, dry weight, and stem height) were measured on seedlings.

In a second experiment, pepper seedlings with nine unfolded leaves were transplanted into containers and grown in soilless production systems in a passively ventilated greenhouse. Seedlings were transplanted in March, three days after each seedling had been artificially infested with two female broad mites. Treatments included a) release of either 2 or 4 *N. californicus* per plant at either -3, +3, or +7 DFI, b) five weekly micronized-sulfur sprays (80% sulfur at a rate of $3.4 \text{ kg}\cdot\text{ha}^{-1}$) initiated at either -3 or +3 DFI, c) infested with no pest management, and d) non-infested control. Within the greenhouse, plots were isolated with vertical polyethylene film barriers. Top plant canopy leaves were sampled weekly for mite recovery, and early marketable yield of red fruit was harvested. Both experiments were arranged as randomized complete block designs.

RESULTS & DISCUSSION.

In the first experiment, undamaged 42-days-old seedlings were produced when two predators per seedling were released preventively (up to 24 d before seedlings were infested with *P. latus*) (Fig. 1). Growth variables in seedlings that had two predators released on the same day of the infestation, or before the day of the infestation, were not significantly different from seedlings that had not been infested with *P. latus*. However, these undamaged seedlings, which still hosted *N. californicus* at 42 d after seeding, also hosted from 3 to 13 *P. latus*, and were at prey:predator ratios < 7:1 in whole seedlings. It is likely that *N. californicus* could maintain the pest at non damaging levels after seedlings from the latter scenario are transplanted in a greenhouse. However, higher predator release densities than two *N. californicus* per seedling may be needed to obtain pest-free seedlings in transplant flats with widespread infestations of *P. latus*.

Releasing two *N. californicus* per seedling as a curative control strategy (within a time as short as 9 days after an initial infestation with two *P. latus*) in nursery trays was not effective for producing undamaged or broad mite-free transplants (Fig. 1). Growth variables in curative releases were up to 60% less than in seedlings that had not been infested with *P. latus*. At 42 d after seeding, severely damaged seedlings averaged up to 5000 *P. latus* cumulative mite-days. Seedlings with preventive releases had generally less than 100 *P. latus* cumulative mite-days for growth variables to be reduced 5% or less with respect to measurements in non-infested seedlings, and showed no visual symptoms of mite damage. Peaks of up to 350 *P. latus* per seedling were measured in pest-predator scenarios where *N. californicus* were released 9 to 24 days after the initial *P. latus* infestation. In these curative releases of the predator, *P. latus* populations decreased by day 42 after seeding and were in a range from 16 to 226 *P. latus* per seedling.

In the second experiment, serious plant damage occurred where broad mites were left uncontrolled, within a month after transplanting infested seedlings into the greenhouse (Fig. 2). No marketable fruit was harvested from plants where *P. latus* was left uncontrolled (Fig. 3). Marketable fruit loss was due to damage caused by *P. latus* to terminal shoots, flowers, and fruits. Yields of red fruits from plants with four predators per plant released at any of the release times (six days before, at, or four days after transplanting), or with sulfur treatments, were not significantly different from the uninfested control treatment (Fig. 4). Releases of two predatory mites per plant led to yields similar to the uninfested control only when introductions were a week before transplanting (Fig. 4), while later introductions led to serious plant damage and significantly lower yields. Pest populations at fruit harvest were nil in all plants treated with *N. californicus*. During the 88-day crop, releases of *N. californicus* that were effective had ≤ 120 *P. latus* cumulative mite-days on the top canopy leaves (averaging 1 mite/leaf-cm²), while top leaves from plants with no *P. latus* control reached up to 1500 *P. latus* cumulative mite-days. With sulfur sprays, broad mite populations on top leaves were kept at low numbers (≤ 2 mites/leaf-cm²) but mite populations resurged after the spray program was interrupted (after fifth spray).

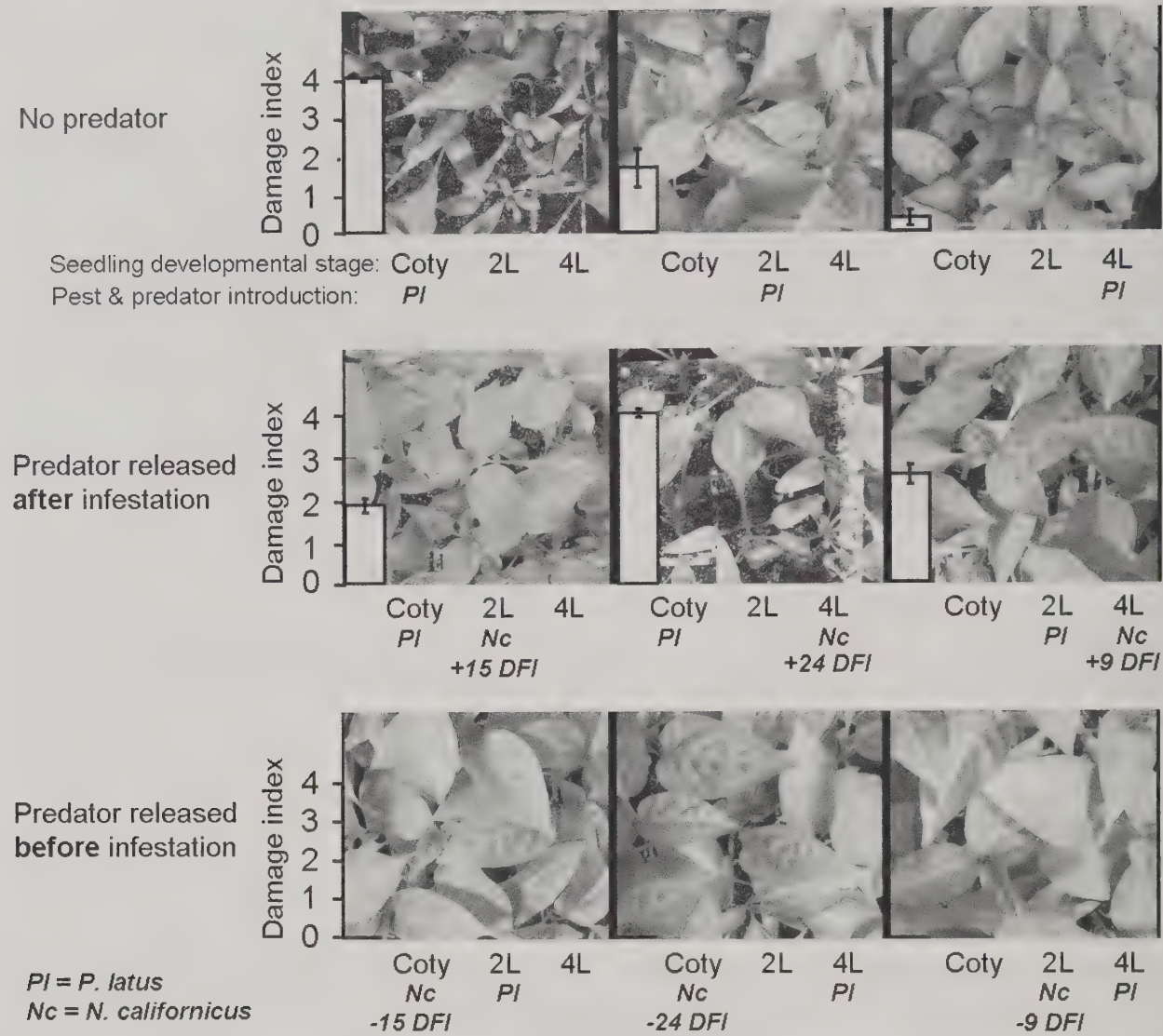


Fig. 1. Damage levels in pepper seedlings 42 d after seeding in selected scenarios where two *Polyphagotarsonemus latus* and two *Neoseiulus californicus* were introduced at different times during seedling development. Developmental stages of non infested seedlings: cotyledons unfolded (Coty), two leaves unfolded (2L), and four leaves unfolded (4L). Days from initial *P. latus* infestation (DFI). Damage scale ranged from 0 (no visual damage) to 4 (necrosis of terminal shoot leaves).



Fig. 2. Pepper plants treated with and without *Neoseiulus californicus* a month after they were transplanted in a greenhouse. (LEFT) Each plant was infested with two *Polyphagotarsonemus latus* 3 d before transplanting but had two *N. californicus* released 6 d before transplanting, and (RIGHT) each plant was infested with two *P. latus* 3 d before transplanting and the pest was left unchecked.



Fig. 3. Fruit on pepper plants treated with and without *Neoseiulus californicus* for an early *Polyphagotarsonemus latus* infestation. Plants (88 days after transplanting) with fruit attached and leaves removed. (LEFT) Plant was infested with two *P. latus* 3 d before transplanting but had two *N. californicus* released 6 d before transplanting, and (RIGHT) Plant was infested with two *P. latus* 3 d before transplanting and the pest was left unchecked (all fruit were damaged and unmarketable). Reference line is 50-cm tall.

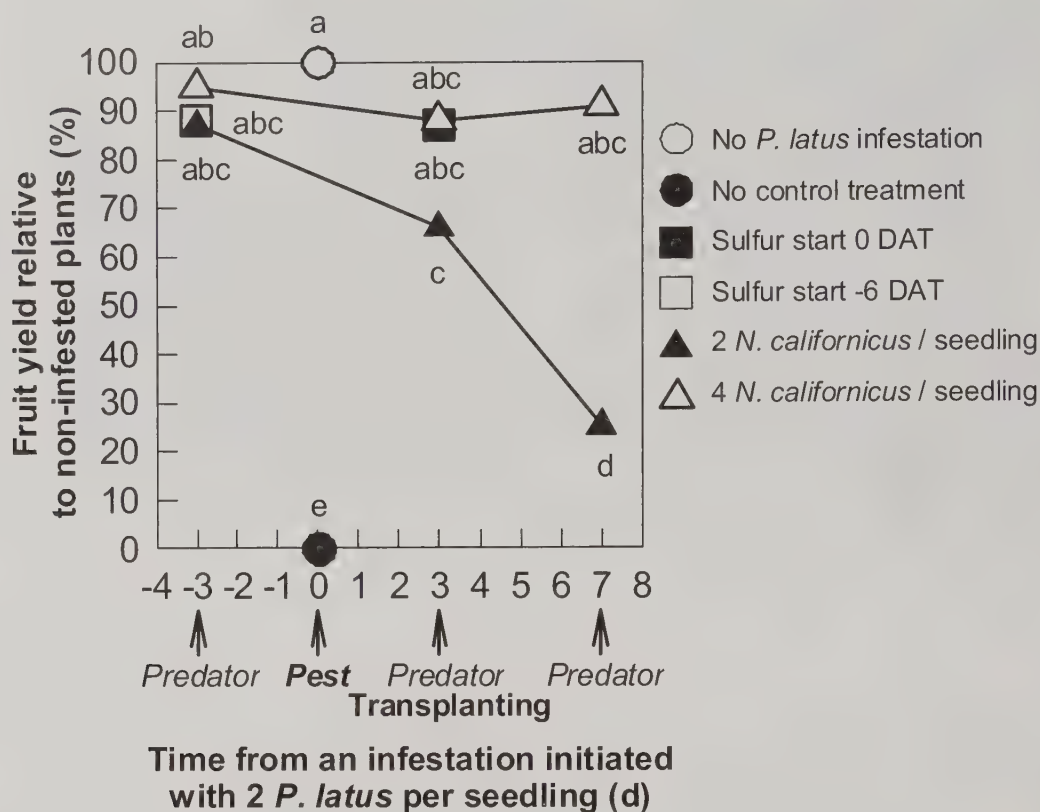


Fig. 4. Marketable fruit yield (88 days after transplanting, DAT) in greenhouse-grown bell pepper plants that were infested with two *Polyphagotarsonemus latus* (–3 DAT) and had the pest managed with a) *Neoseiulus californicus* (released at either –6, 0, or 4 DAT, at predator release densities of two or four per plant), b) sulfur (5 weekly sprays started at either –6 DAT or 0 DAT). Yield was 100% in non infested plants and 0% in infested plants with no pest management. Letters for comparisons are based on Tukey-Kramer test for mean separation.

In tropical and subtropical regions, *P. latus* infests pepper crops at any time during the crop development. Pepper crops are most vulnerable when early crop infestations occur (de Coss-Romero & Peña, 1998) and pest management is delayed because the pest is unnoticed. In pepper seedlings grown in nursery flats as well as in young plants grown in fruit production greenhouses, a curative release of two *N. californicus* per plant once symptoms were observed on top leaves were not effective for avoiding transplant damage or yield reduction. However, initial broad mite infestations in pepper crops grown in greenhouses could effectively be controlled with *N. californicus* released at early seedling developmental stages in the nursery and at transplanting in the fruit production greenhouse. Both *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) (Weintraub *et al.*, 2003) and *N. californicus* are phytoseiids that are commercially reared in many countries and can be used to manage broad mite infestations in greenhouse-grown pepper crops. Released on transplants, *N. californicus* may control early infestations of broad mites and two-spotted spider mites, and become established on the transplanted crop. In production regions where *N. californicus* is available, this predator could be an important pest management tool for growers who intend to produce pesticide-free crops, crops with a minimum use of pesticides, or crops that follow organic production standards.

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THE EFFECT OF GROUND COVER MANAGEMENT ON THE BIOLOGICAL CONTROL OF *TETRANYCHUS URTICAE* (ACARI: PROSTIGMATA) IN CLEMENTINES

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ABSTRACT.

Fruit scarring caused by *Tetranychus urticae* Koch is one of the main pest problems faced by Spanish clementine growers as it can completely downgrade the quality of the fruit. Because of the polyphagous nature of this mite, we hypothesized that ground cover management could affect the dynamics of both *T. urticae* and its Phytoseiidae natural enemies and therefore modulate damage. A previous study, where the acarofauna associated to weeds appearing in citrus orchards was characterized, showed that Poaceae presented the most favorable Phytoseiidae: *T. urticae* ratio from all weed species considered. Therefore we compared the effect of a sown cover of *Festuca arundinacea* (Poaceae) to traditional alternatives. Mite populations have been monitored since spring 2006 in four different commercial citrus orchards where three ground management systems have been established in 1 ha plots: (a) a wild cover, (b) a sown cover of *F. arundinacea* and (c) bare soil. As expected, the sown cover of *F. arundinacea* has resulted in the lowest populations of *T. urticae* on the trees. The mechanisms explaining these results could be related both to the composition of the beneficial acarofauna associated to the different covers and to the selection of host-feeding specialist strains of *T. urticae* in the *F. arundinacea* cover.

INTRODUCTION.

The highly polyphagous and cosmopolitan two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) can be an important pest of citrus (Aucejo *et al.* 2003). Mite infestations on clementine mandarins result in chlorotic spots on leaves which can drop prematurely and, more importantly, in fruit scarring. Spain is the largest exporter of citrus for the fresh market worldwide, and injured fruit have no commercial value (Ansaloni *et al.* 2008).

Reasons leading to *T. urticae* infestations in citrus are being unveiled. Stress factors, such as water stress, heat and salinity, and some cultural practices, such as pesticide use, weed management, or fertilization, are related to damage (Ripollés *et al.* 1995, Quirós-González 2000, Aucejo *et al.* 2003, 2004, Ansaloni *et al.* 2008). Tetranychidae usually reach damaging levels only when their natural enemies, mostly phytoseiid mites, are rare (Barbosa, 1998) and in this scenario, the management of the citrus ground cover, which acts as a reservoir of both phytophagous and predatory mites (Aucejo *et al.* 2003) can be crucial. Spanish citrus trees are usually grown on bare soil by means of herbicide treatments (Gómez de

Barreda 1994). This strategy can lead to massive migrations of *T. urticae* up to the tree, as observed in other agrosystems (Kim & Lee 2003, Hardman *et al.* 2005), and result in negative effects on phytoseiids (Pratt & Croft 2000). Ground cover management is an important component of any conservation biological control strategy (Barbosa 1998), especially when mites on perennial systems, such as citrus, are involved (Nyrop *et al.* 1998). A study carried out previously in different citrus commercial orchards in Spain (Aucejo *et al.* 2003) showed that grasses from the family Poaceae harbored the lowest populations of *T. urticae* in relation to Phytoseiid mites. Based on these results, we decided to investigate the dynamics of both *T. urticae* and its Phytoseiid natural enemies under three different ground cover management strategies: bare soil, a wild cover and a cover sown with *Festuca arundinacea* Schreb.(Poaceae).

MATERIALS AND METHODS.

The population dynamics of both spider mites and Phytoseiids, were studied in four commercial clementine mandarin orchards (cv. Celementina de Nules grafted on Citrange Carrizo) located in eastern Spain [L'Alcúdia, LA (39° 10.62' N; 0° 33.32' W; h: 25 m), Bétera, BÉ, (39° 35.10' N, 0° 24.40' W; h 120 m), Lliria, LL, (39° 43.67' N; 0° 35.14' W; h: 164 m) and La Pobla de Vallbona, PV, (0° 30.51' N; 39° 38.10' W; h: 125 m)]. Two of these orchards were organic (LA and BE), another one followed IPM guidelines (LL) and the last one was managed with regular applications of pesticides (PV). At each location, we studied three ground management systems, which occupied about 1 ha each. The first system was bare soil by means of herbicide applications (LL, PV) or labor (LA, BE). The second one consisted of a homogeneous cover of *F. arundinacea*, and finally, the third system consisted of the existing wild cover. In these two cases, the cover was regularly mown and grass clippings left in place. The four commercial orchards were sampled fortnightly for almost two years, from March 2006 to December 2007. At each visit, we collected 100 leaves from the trees on each management system, as well as 100 grams of the cover crop.

Mites and other arthropods were extracted in Berlese funnels and preserved in ethanol. Afterwards, both tetranychid and phytoseiid mites were separated under binocular microscope. These mites were cleared and mounted for microscope observation. Tetranychid mites were determined based on the morphology of the male edeagus (Ferragut & Santonja 1989) whereas phytoseiids were determined by observation of dorsal and ventral chetotaxy and according to morphology of female spermathecae (García Marí *et al.* 1990).

Actual mite counts were transformed to cumulative mite-day values for each treatment and year. These values were compared by ANOVA. The Duncan test was used for mean separation at $P = 0.05$. The software package Statgraphics (Manugistics Inc., MD, USA) was used for the analyses.

RESULTS.

The four experimental orchards were sampled 46 times each. A total of 556 leaf samples and 368 and ground cover samples were collected. 9,200 mite specimens were extracted from these samples. 7,130 mites were Tetranychidae (*Panonychus*

citri (Mc Gregor), *Tetranychus evansi* Baker & Pritchard and *T. urticae*), 612 were Phytoseiidae (*Amblyseius andersoni* Chant, *A. bicaudus* Wainstein, *Euseius stipulatus* Athias-Henriot, *Neoseiulus barkeri* Hughes, *N. californicus* McGregor, *Phytoseiulus persimilis* Athias-Henriot and *Typhlodromus phialatus* Athias-Henriot) and the remaining 1,458 belonged to other taxa.

Fig. 1 shows the population dynamics of both Tetranychid and Phytoseiid mites at the BE orchard, which can be considered as a paradigm of what was found at the other three locations. Important peaks of *T. urticae* were observed both on the trees and the ground cover for the wild cover, whereas *T. urticae* peaks observed on the *F. arundinacea* cover, even if bigger than those observed on the wild cover, did not result in peaks on the associated trees. Similarly, *P. citri* peaks on trees were never associated to those found on *F. arundinacea*.

Mean, maximum and cumulative mite-days (CMDs) counts for both *T. urticae* and Phytoseiidae are shown in Tables 1 and 2 for 2006 and 2007, respectively. Prior to analyzing the differences among the different types of cover and location, possible differences between years were considered. No differences were observed ($P > 0.05$) except for *T. urticae* on the cover ($F_{1; 15} = 7.41$; $P = 0.0165$). Therefore, data from 2006 and 2007 were further pooled for analyses except for that particular case.

Orchard location did not significantly ($P > 0.05$) affect *T. urticae* CMDs values on either the cover or the tree. However, the cover management affected *T. urticae* CMDs on the trees ($F_{2; 23} = 5.11$; $P = 0.0248$). The highest values were reached on the trees grown on the wild cover, whereas there were no significant differences between those grown on the *F. arundinacea* cover and on bare soil. Contrarily, the CMDs of *T. urticae* on the ground covers assayed were not significantly different from each other ($P > 0.05$).

Both orchard location and the management of the ground cover significantly affected the Phytoseiidae CMDs values both on the ground cover and the trees ($P < 0.0005$). The interaction between these two factors was not significant for ground cover values ($P = 0.4594$), but it was for values registered on the trees ($P = 0.0087$). The highest Phytoseiid records on the ground cover were found at the organic orchard at BE and the lowest at the chemical orchard at PV. The other two orchards were not significantly different from each other and showed intermediate Phytoseiid values. Likewise, the highest Phytoseiidae values on the ground cover corresponded to the *F. arundinacea* treatment. The high Phytoseiid populations on the trees grown on the wild cover at BE, made this treatment significantly different from the other two. However, these differences disappeared at the other three locations and Phytoseiid CMDs on the trees were very similar irrespective of the ground cover where they were grown.

Mite composition during 2006 and 2007 is shown in Fig. 2. The predominant Phytoseiidae species on the trees was *E. stipulatus*. This species represented about 3/4 of total Phytoseiids found except for the *F. arundinacea* cover, where it barely exceeded 1/2. Similarly, this Phytoseiid was predominant on the wild cover, but its prevalence was not so clear on the *F. arundinacea* cover, where other Phytoseiid species were as abundant as *E. stipulatus*. Furthermore, *A. andersoni* was found on this cover only. *Tetranychus urticae* was the most abundant species both on the

trees and the ground covers in 2006. However, in 2007, *T. evansi* displaced this species on the wild cover. The highest Phytoseiidae: *T. urticae* ratios were found on the *F. arundinacea* cover (mean: 0.67 ± 0.14 vs 0.37 ± 0.07 in the wild cover), whereas there was no clear trend for the same ratio on the trees (mean: 0.33 ± 0.10 , 0.32 ± 0.09 and 0.26 ± 0.08 , for the wild cover, the *F. arundinacea* and the bare soil treatments, respectively).

DISCUSSION.

Our results show that trees grown on the wild cover had the highest populations of *T. urticae*. Therefore, these trees were most likely to be exposed to its damage. Both the *F. arundinacea* and bare soil resulted in lower *T. urticae* populations on the trees and therefore reduced risks associated to this mite. However, *T. urticae* populations on the wild cover and on *F. arundinacea* were not significantly different and this result poses some questions.

The selection of *F. arundinacea* was based on previous results (Aucejo *et al.* 2003) where Poaceae were considered as poor hosts for *T. urticae*. Mite populations on *F. arundinacea* have not been significantly different from those on the wild cover. If we take for granted that, as in other systems (McMurtry and Croft 1997, Barbosa 1998, Kim and Lee 2003, Hardman *et al.* 2005) *T. urticae* populations on the ground and on the tree are connected, there must be something that does not allow mites on *F. arundinacea* to successfully move up and establish on the trees. The mechanisms explaining these results could be related both to the selection of a host-feeding specialist strain of *T. urticae* in the *F. arundinacea* cover and to the composition of the beneficial acarofauna associated to the different covers.

On the one hand, *T. urticae* is highly polyphagous and can easily adapt to new hosts plants (Agrawal 2000; Fry 1989, Gould 1979, Navajas 1998). However, host-feeding specialist strains of this mite have been described (Gotoh *et al.* 1993). Therefore, this species could be a mixture of actually polyphagous individuals or a collection of specialists (Navajas 1998). The latter hypothesis could partly explain our results. *Tetranychus urticae* has a very short generation time and a high fecundity in combination with *Wolbachia*-related factors of cytoplasmic incompatibility (Stouthamer *et al.* 1999). These mechanisms could favor the occurrence of this hypothesis. In fact, field studies carried out in Greece, demonstrated that *T. urticae* populations from lemon trees collected in different orchards in different locations were genetically closer than those collected on 11 additional hosts at the same locations (Tsagkarakou 1998). Should this specialization phenomenon occur in our orchards, the specialist strain occurring on *F. arundinacea* would be unable to successfully colonize the trees and vice-versa. However, the wild cover (where 76 different species belonging to 27 different families occur) would ensure the conservation of different host-feeding strains that would allow successful colonization of the tree when weeds were either killed or mowed.

On the other hand, the *F. arundinacea* cover sheltered the highest populations of Phytoseiidae and this could result in a better control of *T. urticae* and a barrier for this mite to move up to the trees. Contrary to what could be expected from a monovarietal cover, Phytoseiidae diversity on *F. arundinacea* was higher than on the wild cover. *Euseius stipulatus* was the predominant species both on the trees, as

previously described by García-Marí *et al.* (1999), and on the ground cover. However, its predominance on the tree grown on *F. arundinacea* was not as strong as on the other systems. This generalist predator is a key mortality factor against *P. citri*, but can not be considered as a good predator of *T. urticae* (Abad-Moyano *et al.* 2008). The higher presence of *T. phialatus* and especially of *N. californicus* and *P. persimilis* on *F. arundinacea* and its associated trees could also account for our results.

This study clearly shows that the wild cover should not be encouraged in citrus. Both bare soil and *F. arundinacea* reduced the presence of *T. urticae* on the trees and therefore the risks associated to this phytophagous mite. However, the bare soil situation in our study is not exactly what can be found in commercial orchards, where weeds are not removed every two weeks. In that case, weeds can be found in the orchard, and if herbicides are used, a selection of good *T. urticae* hosts can occur (Aucejo *et al.* 2003). Therefore, *F. arundinacea* appears as the most suitable alternative. However, further research aimed at elucidating the trophic relationships occurring in the citrus system is needed.

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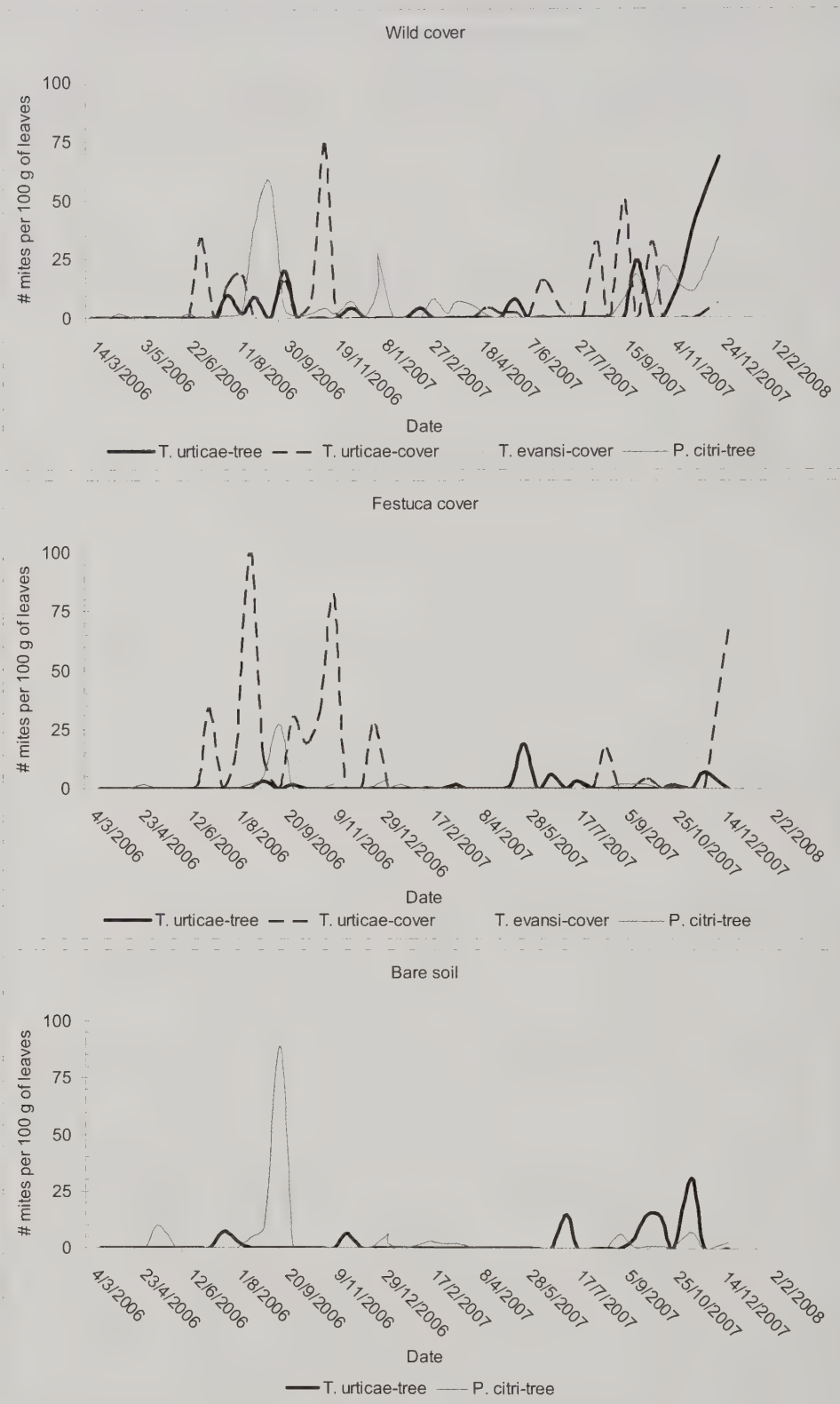


Fig 1.a. Population dynamics of **Tetranychidae** in the Bétera clementine commercial orchard.

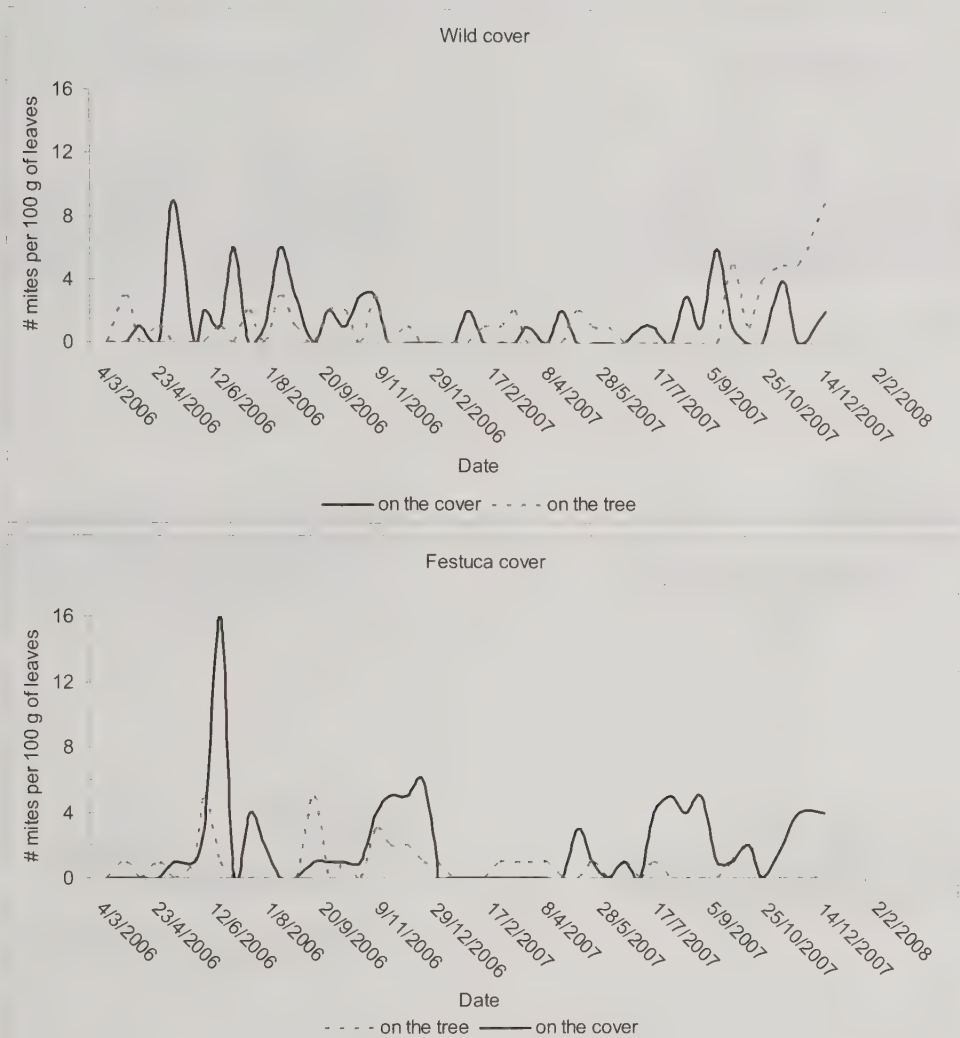


Fig 1.b. Population dynamics of **Phytoseiidae** in the Bétera clementine commercial orchard.

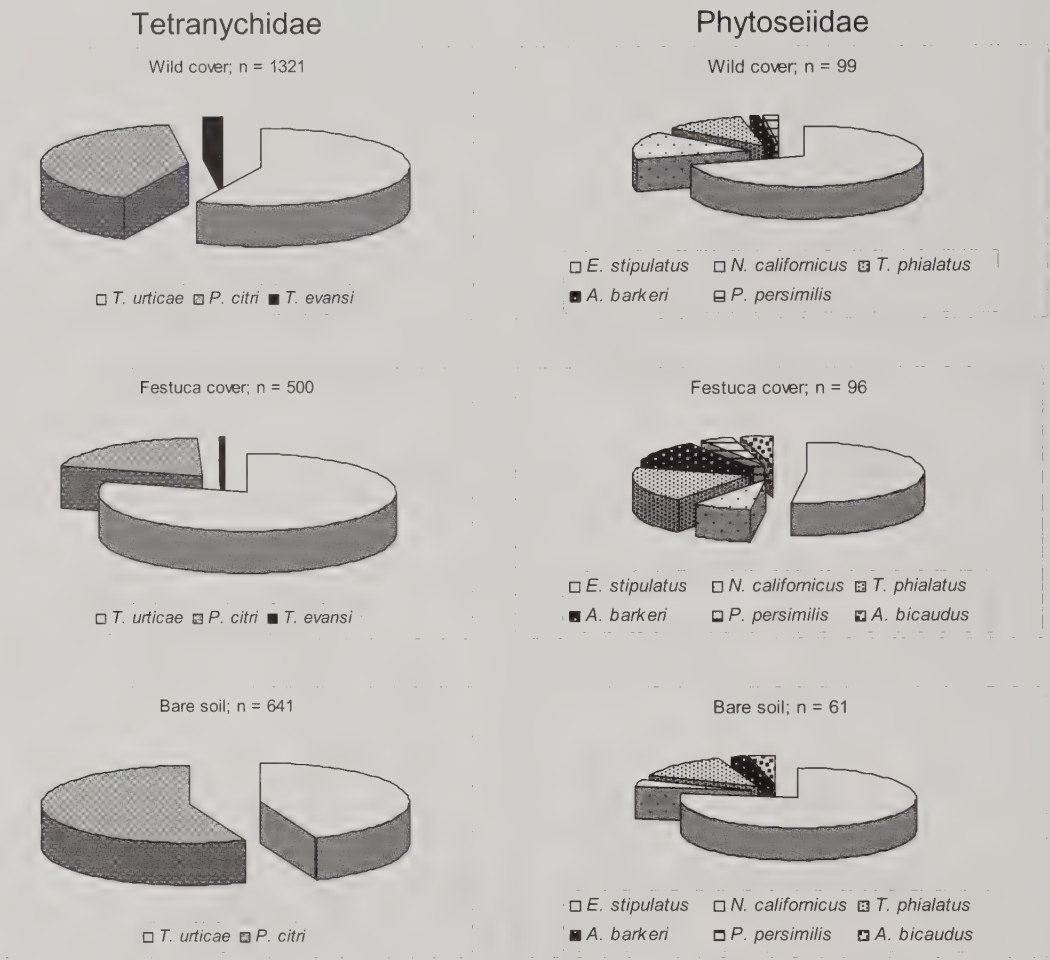


Fig 2a. Specific composition of Tetranychidae and Phytoseiidae in 2006 and 2007 **on the trees** grown on the three management systems considered.

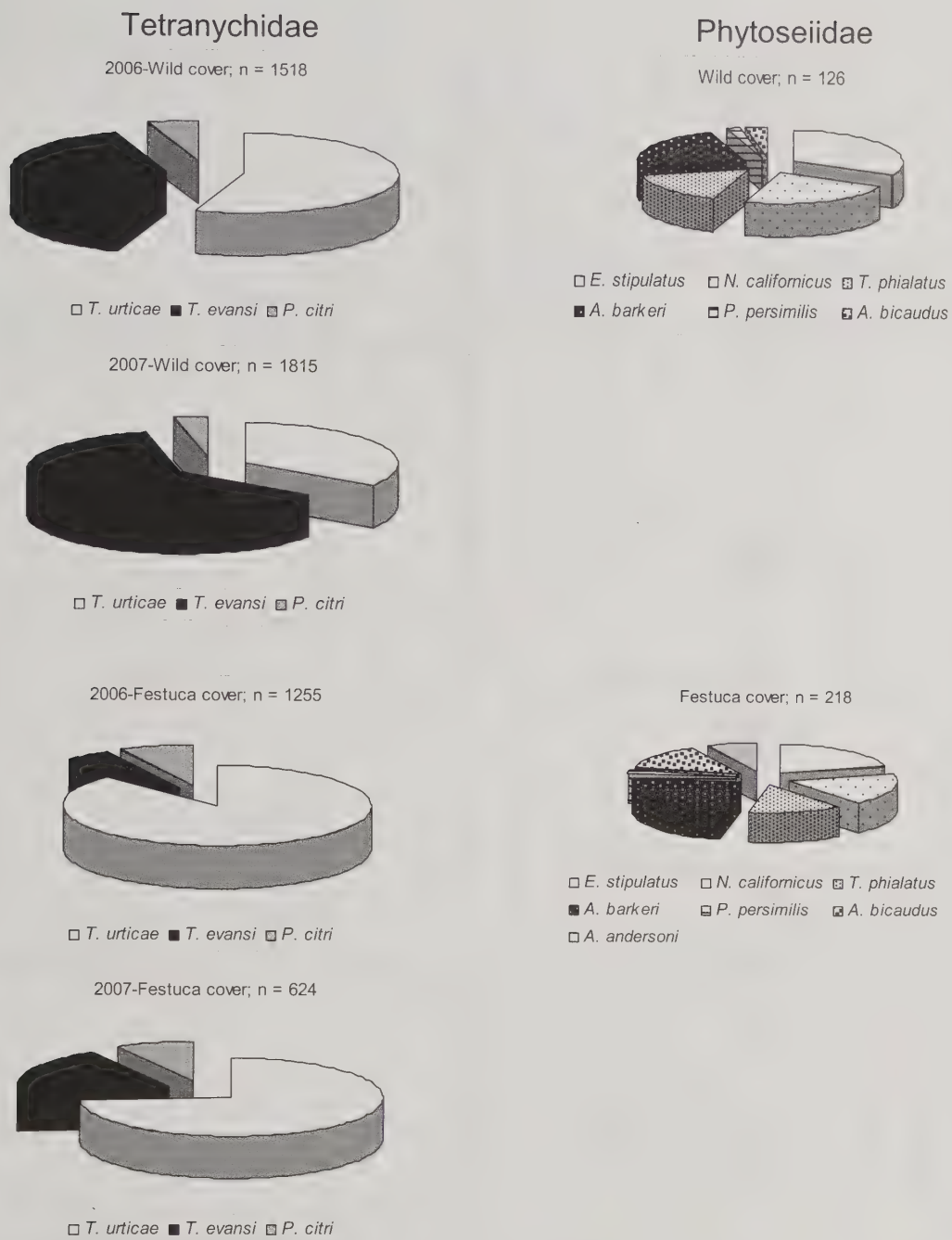


Fig 2b. Specific composition of Tetranychidae and Phytoseiidae in 2006 and 2007 **on the ground cover** of the three management systems considered.

Table 1. Mean (M ± EE), maximum (Max) and cumulative mite days (CMDS) of *T. urticae* and Phytoseiidae on tree and ground cover registered on each ground cover management system in 2006 for the orchards at l'Alcúdia, Bétera, Llíria and La Pobla de Vallbona.

	Wild cover						Festuca cover						Bare soil					
	Ground cover			Tree			Ground cover			Tree			Tree			Tree		
	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM
L'ALCÚDIA																		
<i>T. urticae</i>	6.23 ± 2.41	47	3305.5	5.05 ± 2.10	38	2280	9.07 ± 3.28	62	4972	1.35 ± 0.58	10	699	1.04 ± 0.55	13	556.5			
Phytoseiidae	0.54 ± 0.19	4	290	0.77 ± 0.34	7	421.5	0.46 ± 0.18	4	274.5	0.39 ± 0.19	4	229.5	0.19 ± 0.10	2	91			
BÉTERA																		
<i>T. urticae</i>	6.39 ± 3.14	74	3342	2.23 ± 0.90	20	1014	13.77 ± 5.06	101	7344	0.19 ± 0.14	3	111	0.65 ± 0.37	7	330			
Phytoseiidae	1.54 ± 0.46	9	418	0.77 ± 0.21	3	836	2.00 ± 0.67	16	1062	0.96 ± 0.28	5	518.5	0.81 ± 0.24	4	404			
LLÍRIA																		
<i>T. urticae</i>	5.12 ± 1.58	25	2785.5	4.86 ± 1.89	43	2212	6.42 ± 0.28	34	3363	1.23 ± 0.90	22	678.5	2.19 ± 1.08	24	1194			
Phytoseiidae	0.58 ± 0.26	6	318	0.23 ± 0.10	2	124	0.61 ± 0.21	4	339.5	0.27 ± 0.11	2	143	0.23 ± 0.10	2	127			
LA POBLA DE VALLBONA																		
<i>T. urticae</i>	11.31 ± 4.61	92	6315.5	2.18 ± 1.31	33	1107	8.92 ± 3.09	52	3317	0.81 ± 0.59	15	314.5	0.00 ± 0.00	0	0			
Phytoseiidae	0.62 ± 0.22	4	329.5	0.00 ± 0.00	0	0	1.23 ± 0.39	8	453	0.19 ± 0.10	2	65.5	0.00 ± 0.00	0	0			

Table 2. Mean (M ± EE), maximum (Max) and cumulative mite days (CMDs) of *T. urticae* and Phytoseiidae on tree and ground cover registered on each ground cover management system in 2007 for the orchards at l'Alcúdia, Bétera, Llíria and La Pobla de Vallbona.

Wild cover										Festuca cover										Bare soil									
Ground cover					Tree					Ground cover					Tree					Tree					M ± EE				
M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM	M ± EE	Max	ADM
L'ALCÚDIA																													
<i>T. urticae</i>	3.21 ± 1.43	22	1732.3	2.50 ± 1.22	26	1816	5.08 ± 2.22	39	2789.5	2.50 ± 1.34	27	1253	0.63 ± 0.35	6	273														
Phytoseiidae	0.29 ± 0.09	1	148	0.38 ± 0.16	3	200.5	1.33 ± 0.39	6	792	0.33 ± 0.14	3	171	0.29 ± 0.11	2	150.5														
BÉTERA																													
<i>T. urticae</i>	6.06 ± 5.58	50	3207.5	6.60 ± 3.26	68	4038	3.76 ± 2.77	68	1374	1.56 ± 0.82	19	892.5	3.16 ± 1.51	31	1837														
Phytoseiidae	1.96 ± 0.30	6	529.5	1.48 ± 0.47	9	863	1.48 ± 0.36	5	791.5	0.44 ± 0.11	2	224	0.56 ± 0.14	2	290														
LLIRIA																													
<i>T. urticae</i>	7.74 ± 2.33	39	3903.5	1.40 ± 0.91	22	728	3.32 ± 1.24	18	1770.5	1.44 ± 1.29	33	757	1.32 ± 0.55	10	655.5														
Phytoseiidae	0.41 ± 0.13	2	198.5	0.24 ± 0.10	2	112.5	0.96 ± 0.26	6	488.5	0.40 ± 0.19	4	207	0.20 ± 0.11	2	104														
LA POBLA DE VALLBONA																													
<i>T. urticae</i>	6.64 ± 2.19	39	3346	1.08 ± 0.98	25	595	4.48 ± 1.96	50	850	0.04 ± 0.4	1	14.5	0.20 ± 0.14	3	106														
Phytoseiidae	0.60 ± 0.17	3	305.5	0.20 ± 0.08	1	99	1.48 ± 0.31	5	523	0.16 ± 0.09	2	39.5	0.36 ± 0.13	2	181.5														

Anystis baccarum - a potential biocontrol agent in UK apple orchards

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ABSTRACT.

Increasing concern over the use of chemical pesticides is continuing to pressurise apple growers to look for alternative means of invertebrate pest control. The re-discovery of the beneficial predatory mite, *Anystis baccarum*, in UK Bramley orchards offers a potential alternative control component for incorporation into IPM strategies. *Anystis baccarum* has been shown to readily feed upon economically important invertebrate pest species including European fruit tree red spider mite, *Panonychus ulmi*, and show a level of compatibility with chemical pesticides. Mis-identification of this beneficial mite species has resulted in unnecessary pesticide applications being applied within UK apple orchards. Apple growers must be encouraged to be aware of *A. baccarum*, and indeed all predatory fauna, within their orchards and seek to conserve their populations.

INTRODUCTION.

Apple orchards cover c. 27,000 ha in the United Kingdom (Solomon 1992), of which 14,000 ha are dessert varieties, 9,000 ha culinary varieties, and 4,000 ha are for cider making. The major concentration of dessert apples and culinary apple orchards is in the southeastern county of Kent in England. Mostly culinary apples are grown in Northern Ireland. Within the orchards a wide range of predators have been exploited, or have the potential to be exploited, as biological control agents against pests of fruit. These include members of the families Miridae (mirid bugs) (Hemiptera), Anthocoridae (flower bugs) (Hemiptera), Forficulidae (earwigs) (Dermaptera) and several mite families (Solomon 1992).

In relation to invertebrate pest control in Northern Irish apple orchards, on average, 2-3 sprays of insecticide or acaricide per annum are applied. While this is a small expenditure compared with costs of fungicide application, there are several reasons for the avoidance of unnecessary sprays (Cuthbertson & Murchie 2005a): (1) adverse public attitudes to pesticides have intensified in recent years and this has led to a desire by fruit growers to reduce dependence on pesticides, especially broad-spectrum neurotoxic compounds that can adversely affect human health or the environment; (2) unnecessary applications of pesticides can foster pest resistance to the chemical making it useless; (3) unnecessary chemical applications are a waste of money in an increasingly competitive business and; (4) within a comparatively stable ecosystem like orchards, the ability of natural enemies to control pests, completely or partly, is well documented. Loss of natural enemies means that minor pests can become more damaging as the natural constraints on their populations have been removed.

MONITORING PREDATORY FAUNA.

Leaf-dwelling predators such as the mites *Typhlodromus pyri* Scheuten (Mesostigmata: Phytoseiidae) and *Zetzellia mali* (Ewing) (Prostigmata: Stigmaeidae) can be sampled by leaf washing (Zacharda *et al.*, 1988). However, larger predatory species are generally more mobile and cannot be sampled in this way as they disperse when vegetation is disturbed. Various trapping devices have been described by various authors for trapping insect fauna including earwigs, mites and mirids (Helsen *et al.* 1998; McAdam *et al.* 1994; Stewart & Gaylor 1991). Using a selection of trapping devices a study within Northern Irish apple orchards revealed that the predatory mite *Anystis baccharum* (Linnaeus) (Prostigmata: Anystidae), sometimes referred to as the 'whirligig' mite due to its spiral-like running fashion, was the most abundant beneficial species in the orchards (Cuthbertson 2000; Cuthbertson & Murchie 2004a, 2005b). More *A. baccharum* were sampled within Northern Irish orchards than *T. pyri* (Cuthbertson & Murchie 2005b).

DIFFERENTIATION OF ANYSTIS BACCARUM FROM RED SPIDER MITE.

Anystis baccharum (Fig. 1a) is easily spotted on the fruit and foliage of apple trees as it is red/orange in colour, long-legged, and very mobile. If growers are unaware of this species, they may assume that any red mite on the apple trees was the pest species, European fruit tree red spider mite, *Panonychus ulmi* (Koch) (Prostigmata: Tetranychidae) (Fig. 1b) (Cuthbertson 2004, 2005; Cuthbertson & Murchie 2005c). However, certain characteristics enable *A. baccharum* to be identified in the field (Cuthbertson & Murchie 2007a):

- (1) *Anystis baccharum* ranges in size from 1.0-1.5 mm in diameter. This is a lot larger than *P. ulmi*, which when fully grown is about 0.4 mm in diameter.
- (2) *Anystis baccharum* moves rapidly over the branches and foliage of the trees, whereas, *P. ulmi* is relatively sedentary and only found on the under-side of leaves.
- (3) Eggs of *P. ulmi* are visible on the twigs of the trees during the winter months, whereas *A. baccharum* lays eggs under loose bark on the trunk or in the soil surrounding the tree base.

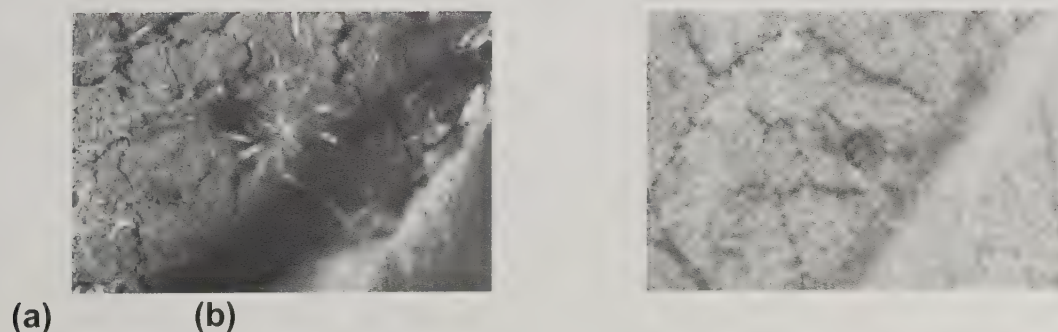


Fig. 1. (a) The predatory mite *Anystis baccharum*; (b) European red spider mite, *Panonychus ulmi*.

PHENOLOGY OF ANYSTIS BACCARUM IN APPLE ORCHARDS.

Anystis baccharum occurs within the orchards almost all year round (Fig. 2), with abundance peaking during spring and early summer (Cuthbertson & Murchie 2004b). This is at the same time as pests such as red spider mite eggs are beginning to hatch and apple rust mite (*Aculus schlechtendali*, (Nalepa) (Prostigmata: Eriophyidae)) are beginning to migrate from their over-wintering sites out onto foliage to feed. Any prey items encountered by *A. baccharum* will be readily devoured. Over-wintering eggs of apple-grass aphid (*Rhopalosiphum insertum*, (Walker) (Homoptera: Aphididae)) and red spider mite, along with apple rust mite, possibly act as a valuable food source to sustain populations of *A. baccharum* over the winter period when other prey items are few (Cuthbertson & Murchie 2006a, 2007b,c).

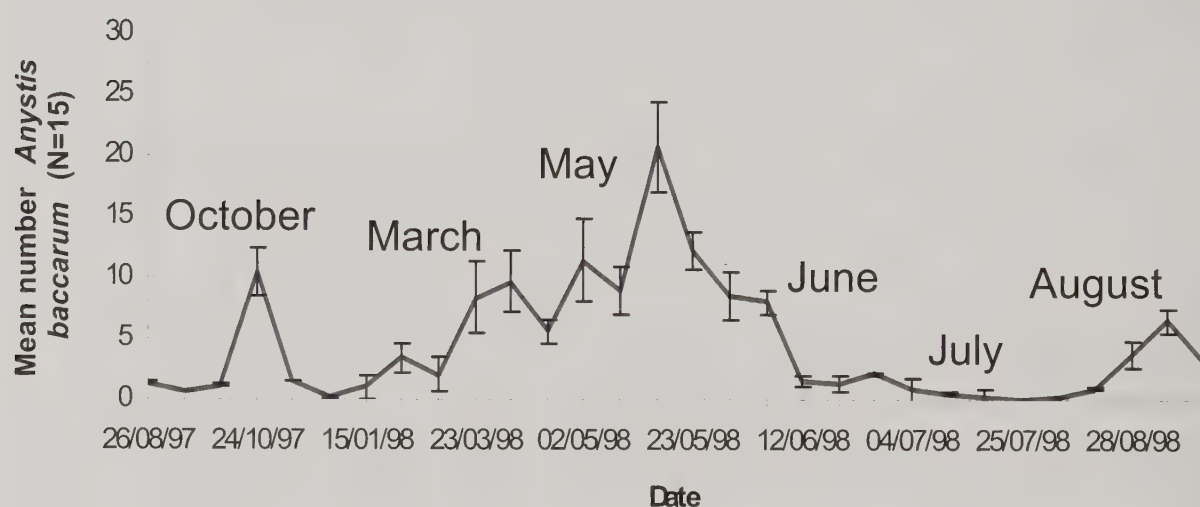


Fig. 2. The phenology of *Anystis baccharum* in a Northern Irish apple orchard.

FEEDING RATE OF ANYSTIS BACCARUM ON ORCHARD PESTS.

Anystis baccharum has been cited as feeding on a range of invertebrate prey (Baker 1967). The mite will readily feed upon any prey item it can over-power (Fig. 3). *Anystis baccharum* is not only a potentially valuable predator in orchards but several other ecosystems, including, blackcurrant bushes in Russia (Lange *et al.* 1974; Livshits & Mitrofanov 1981) and cereal fields in England (El Banhaway *et al.* 1993). In these situations it has offered various levels of invertebrate pest control.

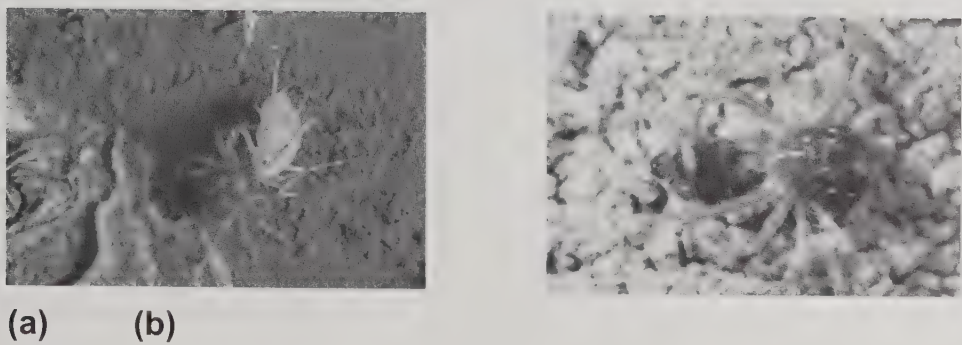


Fig. 3. *Anystis baccharum* attacking and feeding upon (a) aphid and (b) Collembola prey.



Fig. 4. Feeding rate of *Anystis baccharum* on various orchard pests.

Studies undertaken in the laboratory to look at the potential of *A. baccharum* as a bio-control agent against orchard invertebrate pest species have indicated that *A. baccharum* readily feeds upon various apple pests in the laboratory situation, including: *P. ulmi*, *A. schlechtendali*, *R. insertum* and apple-brown mite (*Bryobia rubricollis*, (Scheuten) (Prostigmata: Tetranychidae)) (Fig. 4) (Cuthbertson & Murchie 2004b). The former three species have been reported as being of economic importance within Northern Irish orchards (Mowat & Clawson 1996). *Anystis baccharum* has also been recorded within New Zealand apple orchards as being an important predator of tortricid moth larvae (Baker 1983). It will therefore presumably also feed upon fruit tree tortrix moth (*Archips podana* (Scopoli) (Lepidoptera: Tortricidae)) and *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) larvae within UK orchards (Cuthbertson & Murchie 2005d). *Anystis baccharum* has been shown to be a valuable predator of apple-grass aphid and to offer a degree of apple rust mite control in the orchards (Cuthbertson *et al.* 2003a,b). Exclusion experiments found that rust mite numbers on leaf samples from branches on which *A. baccharum* was

excluded were higher than those from branches where the predator was present (Fig. 5) (Cuthbertson *et al.* 2003a).

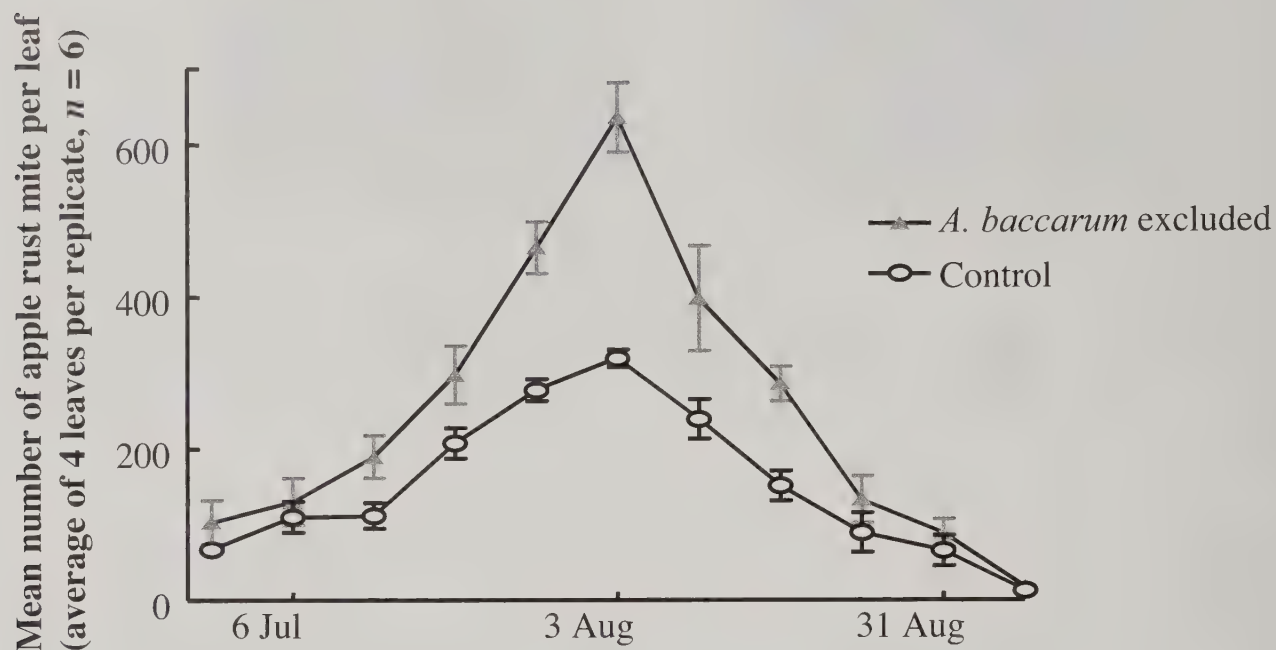


Fig. 5. The effect of excluding *Anystis baccharum* from branches of apple trees on *Aculus schlechtendali* numbers.

COMPATIBILITY OF ANYSTIS BACCARUM WITH CHEMICALS.

Until recently the only information on the impact of chemical pesticides or fungicides on *A. baccharum* was a study from the 1970s by Bushkovskaya (1974), working in Russia. Within the British Isles a more recent study found *A. baccharum* had the potential of being compatible with various chemical fungicides (e.g. dithianon) commonly used for apple scab control (Cuthbertson & Murchie 2003). This same study also showed a link between leaf quality and apple rust mite numbers. Cuthbertson & Murchie (2006b,c) also showed that orchard winter-washes and chemical pesticide applications aimed at controlling invertebrate pests had detrimental effects on *A. baccharum* populations. The beneficial mite was removed by the chemicals and as a result of this, and presumably depletion of other natural enemies, pest populations, such as, *A. schlechtendali* increased within the orchards (Cuthbertson & Murchie 2006d). In Canada, work by Laurin & Bostanian (2007a,b) has shown that dry residues of the fungicides sulphur, captan and myclobutanil are harmless to *A. baccharum* as were the insecticides methoxyfenozide, acetamiprid, imidacloprid and \square pinosad. *Anystis baccharum* therefore offers much potential to be incorporated into IPM strategies within the orchards for invertebrate pest control.

DISCUSSION.

Anystis baccharum is a commonly occurring beneficial mite within Northern Ireland's apple orchards. This mite has been mis-identified by fruit growers as the pest red spider mite, resulting in unnecessary pesticide applications (Cuthbertson

2004, 2005; Cuthbertson & Murchie 2005c). *Anystis baccharum* offers the potential along with the other beneficial species (e.g. *T. pyri*) occurring within the orchards to be incorporated into integrated pest management schemes that would be introduced into the orchards for the control of invertebrate pest species (Cuthbertson & Murchie 2006e, 2007b). Therefore, in the bid to gain a more environmentally sustainable Bramley's Seedling apple production system within Northern Ireland, and the UK as a whole, apple growers need to be made aware of the presence of *A. baccharum*, and indeed all predatory fauna, within their orchards. They should ensure correct identification of *A. baccharum* from *P. ulmi* so preventing unnecessary pesticide applications and take efforts to conserve its populations within their orchards (Cuthbertson & Murchie 2007a). Such an approach has implications beyond UK and Irish orchards, as *A. baccharum* has a widespread and cosmopolitan distribution, including New Zealand, Australia, Canada, USA, Japan and southern Africa (Meyer & Ueckermann 1987).

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BIOLOGICAL CONTROL OF THE TWO SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE* IN CLEMENTINE ORCHARDS IN SPAIN

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ABSTRACT.

The two spotted spider mite, *Tetranychus urticae*, is one of the most damaging tetranychid mites affecting clementine orchards in Spain. Characteristic fruit scarring, which downgrades the commercial value of fresh fruit, is the main damage produced by this species. Natural control of *T. urticae* is insufficient and the extensive use of acaricides has become a common practice. Within the citrus IPM program, much emphasis has been placed on implementing environmentally friendly and sustainable control measures, such as biological control, against this pest. When natural control fails, conservation and/or promotion of the most effective biological control agents found in the field could be an appropriate strategy. From 2004 to 2007 we carried out a detailed survey of arthropod predators associated with *T. urticae* colonies in Spanish clementine orchards. Fourteen predator species belonging to six different arthropod orders (Acari, Coleoptera, Diptera, Hemiptera, Thysanoptera and Neuroptera) were found. Among these natural enemies, two phytoseiid mites, *Neoseiulus californicus* and *Phytoseiulus persimilis*, were selected for inoculative release programs to enhance *T. urticae* control. In the laboratory, both phytoseiid mites achieved a higher intrinsic rate of increase (r_m) than *T. urticae* fed on clementine leaves. Moreover, when promoting them by inoculative releases in nursery conditions, they successfully controlled *T. urticae* throughout the year.

INTRODUCTION.

The two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a cosmopolitan and highly polyphagous phytophagous mite. It can cause economic damage in hundreds of vegetables and fruit trees (Viñuela & Jacas 1998). It is not considered an important pest of citrus trees in the majority of countries where they are cultivated (García-Marí *et al.* 1991; Garrido & Ventura 1993; Smith *et al.* 1997; Talhouk 1973; University of California 1991). However, *T. urticae* has traditionally caused sporadic problems in the citrus of practically all Mediterranean climate producer countries (Bodenheimer 1951; McMurtry 1985; Talhouk 1973). Moreover, during the last decades it has reappeared as pest in Israel (Swirski 1973), Italy (Vacante 1986), Morocco (Hmimina *et al.* 1995), Greece (Souliotis *et al.* 1997) and Spain (Ansaloni *et al.* 2008; Aucejo 2005; Aucejo-Romero *et al.* 2004; Martínez-Ferrer *et al.* 2006; Pascual 2007), specially in the area surrounding the city of Castellón de la Plana (39°59'N; 00°02'W), where over 90% of the Spanish clementine production (1.5 x 10⁶ Tm from 60.000 ha) is located.

The spider mites suck out the cell contents of the lower side of leaves, where they usually inhabit. This results in chlorotic spots, visible on the upper side of leaves. At the end of the summer, *T. urticae* causes characteristic fruit scarring that becomes highly visible during the ripening phase and downgrades fruit (Aucejo-Romero *et al.* 2004; Martínez-Ferrer *et al.* 2006). Furthermore, heavy spider mite infestations combined with water stress can cause massive, sudden leaf dropping (Aucejo-Romero *et al.* 2004; Garrido & Ventura 1993).

In order to keep their clementine orchards unblemished, growers have extensively used acaricides (Aucejo *et al.* 2003). However, chemical control of this pest is problematic (Urbaneja *et al.* 2008). Resistance and pesticide residue problems appear, natural enemies are eliminated, and consequently, outbreaks of *T. urticae* and other pests are induced. In recent years, emphasis has been placed on implementing more environmentally safe measures, such as biological control, to control *T. urticae*.

SURVEY OF NATURAL ENEMIES OF *T. URTICAE*

A necessary step, previous to implementation of biological control measures against *T. urticae* in clementine orchards, is the determination of the natural enemy species composition that co-occur with this pest. Several predatory species had been previously found in association with this pest's webbing: *Stethorus punctillum* Weise (Coleoptera: Coccinellidae), *Conwentzia psociformis* (Curtis) (Neuroptera: Coniopterygidae), *Typhlodromus phialatus* Athias-Henriot (Acari: Phytoseiidae) and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) (García-Marí *et al.* 1991; Ripollés *et al.* 1995; Ripollés & Melia 1980). Nevertheless, citations of these natural enemies were made as part of single observations and their relative importance was unknown. Therefore, from 2004 to 2007 we carried out a detailed survey of arthropod predators of *T. urticae* in Spanish clementine orchards and 14 species of natural enemies were found (Abad-Moyano *et al.* 2008a). These species belonged to several arthropod groups, i.e. Acari, Coleoptera, Diptera, Hemiptera, Thysanoptera and Neuroptera.

The number of phytoseiids (Acari: Phytoseiidae) found per symptomatic leaf was around 0.45. *Euseius stipulatus* (Athias-Henriot) was the dominant species with ca. 69% of the total number of phytoseiids found, followed by *Phytoseiulus persimilis* Athias-Henriot, *N. californicus* and *T. phialatus* (Fig. 1). Eight insect predator species were found in association with *T. urticae*. The number of predatory insects found per symptomatic leaf was around 0.18. The coccinelid *S. punctillum* was the most abundant insect predator (Fig. 2). The gall midge *Feltiella acarisuga* (Vallot) (Diptera: Cecidomyiidae) and the thrips *Scolothrips longicornis* Priesner (Thysanoptera: Thripidae) had not been reported on citrus in Spain previously. The neuropteran *Semidalis aleyrodiformis* Stephens (Neuroptera: Coniopterygidae, and the thread-legged bug *Empicoris rubromaculatus* (Blackburn) (Hemiptera: Reduviidae), had not been reported previously as associated with spider-mite colonies in the field. Both insects are known to feed on *T. urticae*. The relative importance of *P. persimilis* on citrus in Spain is higher than previously thought and, therefore, its expected role as a possible biocontrol agent of *T. urticae* on citrus could be as high as it is in other citrus-growing areas (Smith *et al.* 1997).

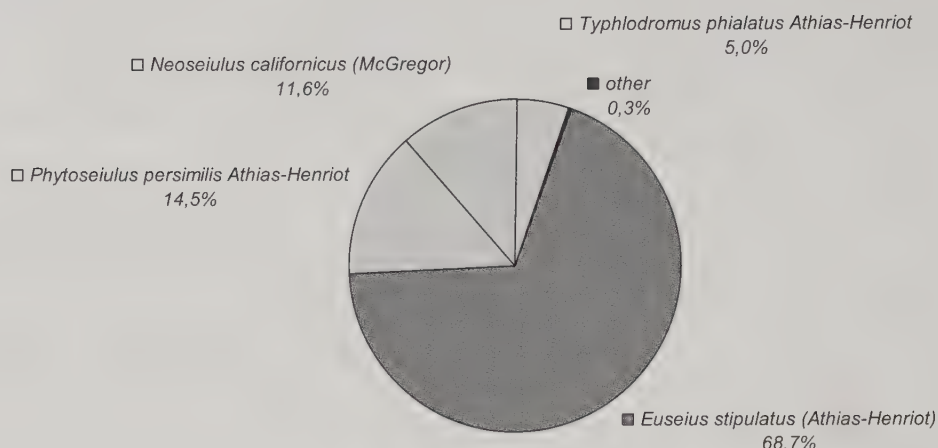


Fig. 1. Relative abundance of phytoseiid species collected on symptomatic leaves with presence of *Tetranychus urticae*, n = 2,617 phytoseiid mites.

The number of phytoseiids extracted from randomly taken leaves by Berlese funnels (0.1 phytoseiids/leaf) was well below that estimated when the sampling method consisted on collecting leaves with spider mite female presence mentioned above. The four most abundant species of phytoseiids detected with the previous sampling method were also found on randomly taken leaves, and their relative abundance followed similar patterns as on symptomatic leaves with presence of spider mites females (Fig. 3).

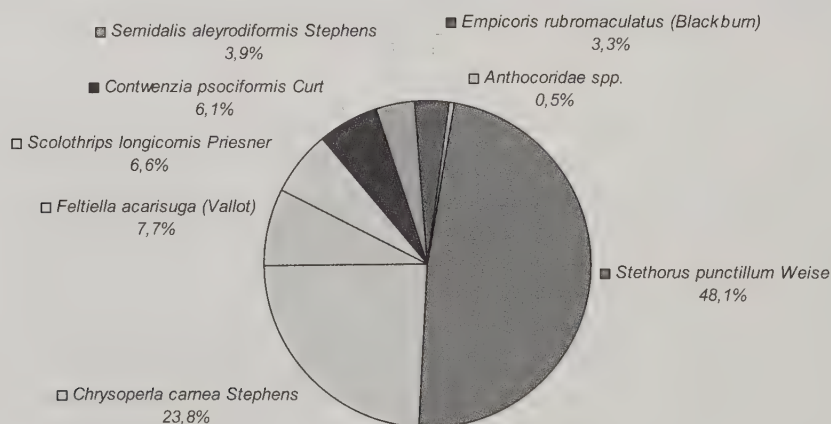


Fig. 2. Relative abundance of insect predators collected on symptomatic leaves with presence of *Tetranychus urticae*, n = 1,022 predatory insects.

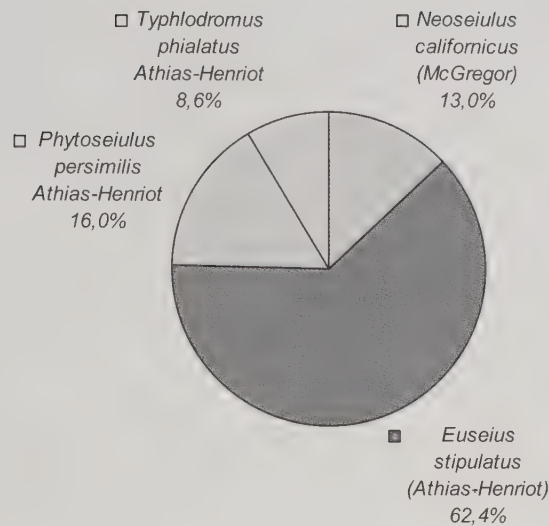


Fig. 3. Relative abundance of phytoseiid mite species collected on leaves randomly taken from trees infested by *Tetranychus urticae*, n = 545 predatory mites.

LIFE HISTORY TRAITS OF THREE PHYTOSEIID MITES ASSOCIATED TO *TETRANYCHUS URTICAE* COLONIES ON INFESTED CLEMENTINE LEAVES.

In clementine Spanish orchards, phytoseiid mites were c.a. 2.5-fold more abundant than predatory insects (Abad-Moyano *et al.* 2008a). Moreover, phytoseiid mites are known to have advantageous traits that make them highly interesting as biological control agents. Some of these traits are: preferential feeding on tetranychid mites, short life cycles, mass-rearability and better ability to thrive at low prey levels than most insects. Indeed, many species of phytoseiids have been successfully used to control spider mite populations in different crops. The 3 most abundant phytoseiid species associated with *T. urticae* colonies in clementines were *E. stipulatus*, *P. persimilis* and *N. californicus*. Life history traits of these phytoseiids preying on *T. urticae* have been widely studied on host plants other than clementine trees. However, the nutritional value of *T. urticae* as well as the foraging behaviour of phytoseiid mite predators may differ from one host plant to another (Castagnoli & Simoni 1999; Cédola & Sánchez 2003; Drukker *et al.* 1997; Krips *et al.* 1999; Popov & Khudyakova 2008; Ragusa & Swirski 1977; Roda *et al.* 2001; Seelmann *et al.* 2007; Skirvin & Williams 1999). Therefore, the life history of *E. stipulatus*, *P. persimilis* and *N. californicus*, when feeding on *T. urticae* reared on clementine leaves was studied (Abad-Moyano *et al.* 2008b), in order to select the best candidate/s for biological control programs against the two-spotted spider mite in clementine orchards. An adequate parameter to use for the selection of candidates for biological control is the intrinsic rate of increase (r_m) (Janssen & Sabelis 1992b). As a rule, candidates with an r_m equal to or higher than that of their prey should have the potential of suppressing local prey populations (Janssen & Sabelis 1992a).

The experiments were performed under laboratory conditions [25°C, 80 ± 5% RH and 16:8 h (L:D)] on clementine leaves and *T. urticae* as prey. *Euseius stipulatus* could not complete its life cycle, whereas *P. persimilis* and *N. californicus* completed it satisfactorily with an estimated intrinsic rate of increase (r_m) higher than the those estimated by other authors for *T. urticae* on clementine leaves [(Aucejo-Romero *et al.* 2004) and (Josep Jacas, Universitat Jaume I, unpublished data)] (Fig. 4). We would expect that the last two predatory mites, and especially *P. persimilis* because of its higher r_m , would satisfactorily control *T. urticae* populations on clementine trees. Nevertheless, it should be taken into account that these values have been obtained under laboratory conditions with unlimited prey supply and other factors and different behavioural patterns of the phytoseiid mites could be involved in these predator–prey systems (Janssen & Sabelis 1992c).

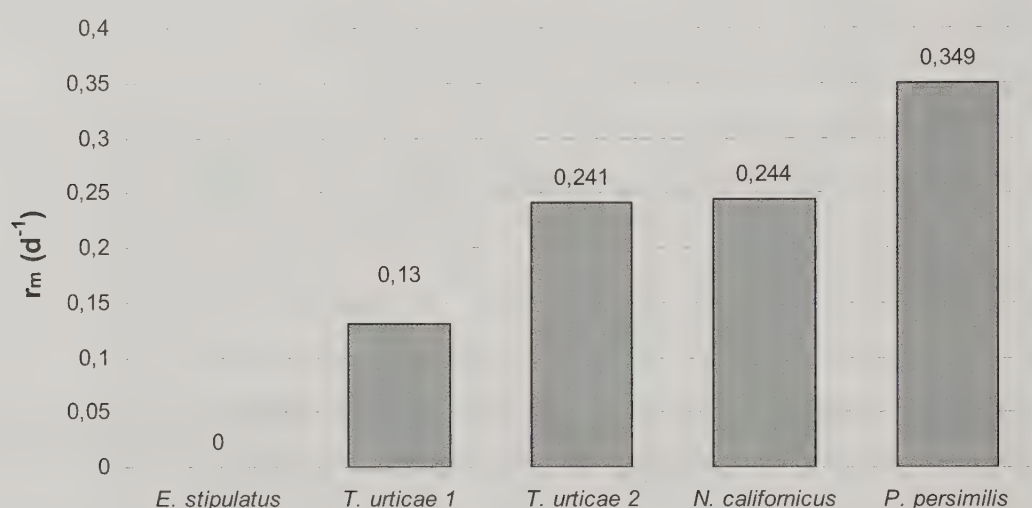


Fig. 4. Estimates of intrinsic rate of increase (r_m) for *Tetranychus urticae* reared on clementine leaves at 25 °C (¹Aucejo-Romero *et al.* 2004 and ²J. Jacas; Universitat Jaume I, Castelló de la Plana, Spain; unpublished data) and for *Euseius stipulatus*, *Phytoseiulus persimilis* and *N. californicus* fed on *T. urticae* reared on clementine leaves at 25 °C.

EFFICACY OF *T. URTICAE* IN SUPPRESSION OF *N. CALIFORNICUS* AND *P. PERSIMILIS*.

Phytoseiulus persimilis and *N. californicus* achieved higher intrinsic rates of increase (r_m) than *T. urticae* fed on clementine leaves, suggesting high biocontrol potential (Abad-Moyano *et al.* 2008b). Since both species are commercially available, their temporary and local promotion by inoculative releases, as has been used in other crop systems, might be a rational control strategy.

In order to assess the efficacy of inoculative releases of *N. californicus* and *P. persimilis* commercial strains (Koppert B.S.) on *T. urticae* suppression on Spanish clementine nurseries, we designed a split-plot experiment (4 plants/rep. x 4 rep.) in semi-field conditions with 2-y-old clemetine plants. We repeated it on the three different periods of the year when the two spotted spider mite represents a major

threat in this kind of plantations in our Mediterranean climatic conditions: spring, summer and autumn. Two different release doses were tested on each experiment (40 and 80 phytoseiids/plant). Plants were artificially infested with 5 *T. urticae* females per plant. One week after first symptomatic leaves were detected, female spider mites were sampled and first phytoseiid mites were released. From first phytoseiid mites release, a weekly sampling was conducted. Non-destructive sampling was conducted by counting, with the naked-eye, *in situ* (without removing the leaf from the plant) the number of *T. urticae* females and the number of visible phytoseiid individuals in four symptomatic leaves, randomly chosen, per plant. In addition, to estimate the damage level on each plant, the number of symptomatic leaves, visible through a ring 20 cm in diameter, was counted per plant. Multiplying this number by the number of spider mite females per symptomatic leaf we had an estimation of the *T. urticae* infestation level measured such as the number of spider mite females per ring as surface unit. We present here the evolution of *T. urticae* infestation level in spring for each treatment considered as an example of the results obtained (Fig. 5) and a photograph of a clementine plant representative of each treatment at the end of the experiment (Fig. 6).

In the control, the spider mite maximum level reached and the time needed to reach this point (week 5, 4 and 10 in spring, summer and autumn with 998.9 ± 149.9 , 554.8 ± 172.3 and 293.8 ± 91.2 spider mite females per ring, respectively) were clearly correlated with mean temperatures during the experiments which were 34.6, 25.3 and 12.5 °C in spring, summer and autumn, respectively. Mean spider mite infestation levels all along the experiment were significantly lower, with respect to control, in all treatments with phytoseiid releases except for low dose of *N. californicus* release in autumn. Accordingly, at the end of the experiments, all phytoseiid releases with significant differences to the control had reduced the *T. urticae* density between 80-100 %. Within species, differences between low and high doses were only found in spring for spider mite infestation level between *N. californicus* release doses.

Mean plant damage was also significantly lower, with respect to control, in all treatments with phytoseiid release except for *N. californicus* release in summer (low dose) and in autumn (low and high dose). In general, plant damage reductions were higher for *P. persimilis*.

CONCLUSIONS.

From the results obtained in the present work, one would expect that inoculative releases of *N. californicus* and/or *P. persimilis* would be sufficient to control this pest, at least under nursery conditions. However, there are several further questions that need to be answered before we know the real potential of controlling *T. urticae* biologically in clementine through promoting phytoseiid action. For instance, possible negative effects of intraguild interactions with the co-occurring predator species, and their impacts on the biological control of *T. urticae* in clementine trees should be determined.

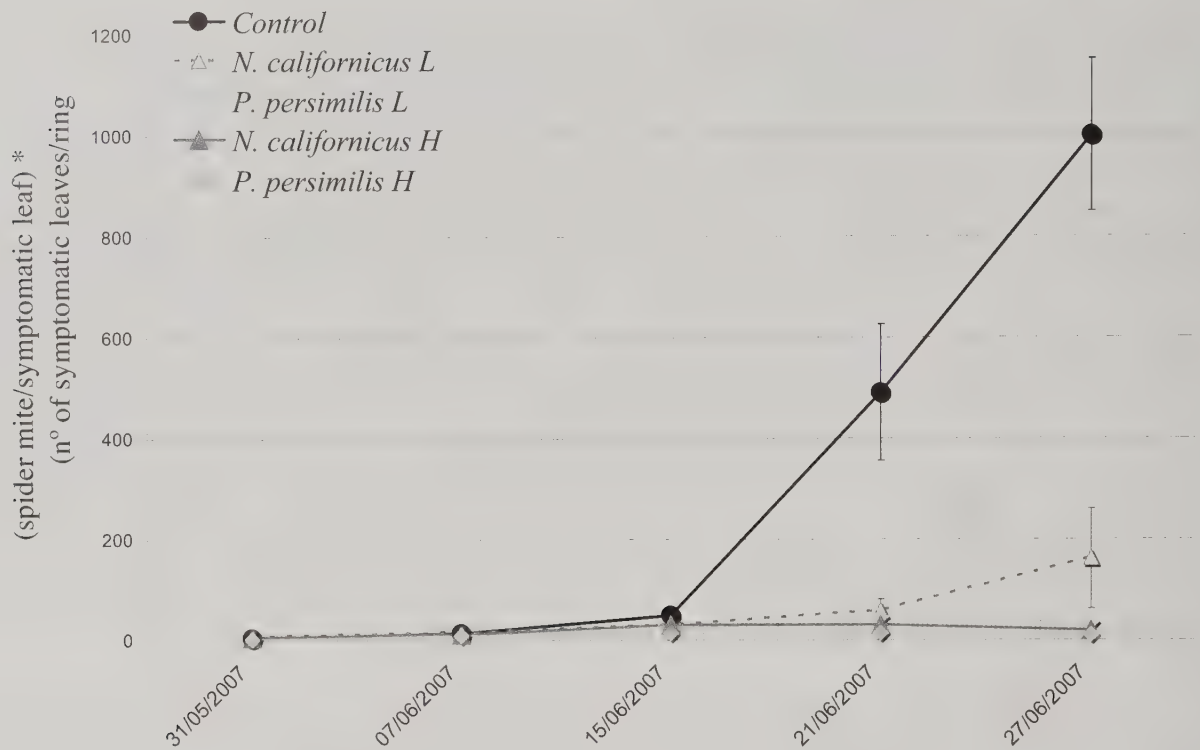


Fig. 5. Number of female spider mites per surface unit ((Number of female spider mites per symptomatic leaf)* (number of symptomatic leaves per ring)) for each treatment in spring.

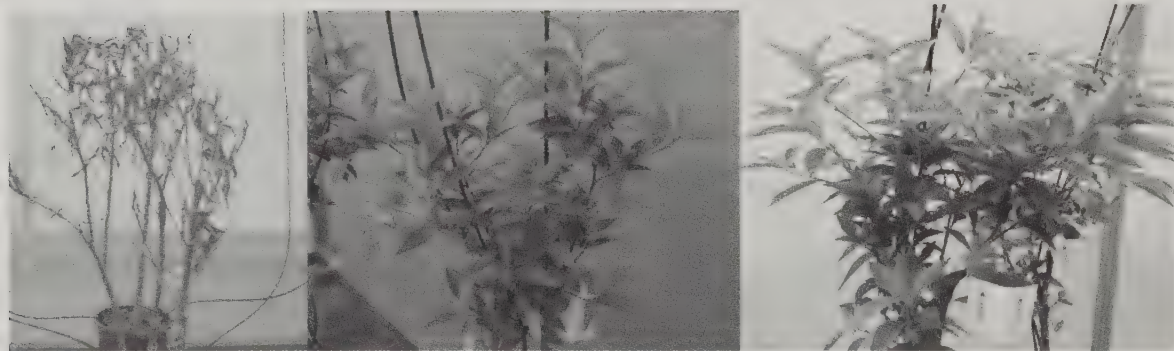


Fig. 6. Aspect, at the end of the experiment, of a clementine plant representative of the (a) control treatment, (b) *N. californicus* release treatment and (c) *P. persimilis* release treatment.

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SESSION 12

ATTRIBUTES OF EXOTIC BIOLOGICAL CONTROL AGENTS: THE GOOD AND THE BAD

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This session was proposed based on an IOBC Global initiative to form a Working Group (WG) on Exotic Biological Control Agents. The goals of the WG are to: (1) assess the characteristics of exotic natural enemies which are considered to be successful biological control agents; (2) assess the characteristics of exotic natural enemies introduced into a country as biological control agents which subsequently become invasive alien species; (3) highlight research areas requiring attention and develop strategies for supporting priority research; and (4) assist in the development of guidelines on assessing environmental benefits and risks of releasing exotic biological control agents to increase cogency of decision making on biological control initiatives. The objective of the session is to generate discussion about what attributes are associated with a desirable biological control agent versus what attributes an undesirable agent possesses. We present a brief introduction to the issue and raise some points about agents that are 'good' and those that are 'bad' including those that have become invasive. This is followed by a series of case studies that outline characteristics of biological control agents that may contribute to successful outcomes or that have resulted in negative or unintended outcomes. We hope that this session will stimulate discussion, generate ideas for new research, and lead to better guidance for efforts to improve assessment of biological control agents.

ATTRIBUTES OF BIOLOGICAL CONTROL AGENTS AGAINST ARTHROPODS: WHAT ARE WE LOOKING FOR?

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ABSTRACT.

Biological control of arthropods is undergoing dramatic change due to heightened awareness of non-target impacts and increasing scrutiny by regulatory agencies. While global trade has contributed to a greater demand for biological control, project funding has remained stagnant or even declined. Thus, to ensure that biological control remains an important option for regulating pests there is a need to provide guidance on what agents are appropriate in a modern, science-based context. Through retrospective analysis it is clear that there are situations where arthropod biological control agents have been successful and other situations where agents failed to have an impact on target pest species, and yet other situations in which biological control agents have backfired, causing more harm than good. By determining those attributes that are associated with effective yet environmentally-benign agents, we may be able to develop recommendations that would be useful for decision-making when selecting biological control agents for study. In contrast, by determining those attributes that are associated with ineffective or environmentally damaging agents, guiding principles can be developed to facilitate avoidance of species with these characteristics.

INTRODUCTION.

Biological control of arthropod pests is an important component of cost-effective, environmentally-benign integrated pest management. Early successes in biological control were dramatic, as exemplified by the vedalia beetle suppression of cottony cushion scale, and resulted in the establishment of programs and infrastructure that focused almost exclusively on foreign exploration for new agents. Generally, if an arthropod biological control agent did not cause damage to economically-important species and no concern was raised about impacts on other non-target species it was considered appropriate for release (Greathead 1986). However, as Greathead (1986) predicted, it is now a requirement that arthropod biological control agents be screened, and assessments of potential environmental impacts be made before release can be approved for use in both augmentative and classical biological control. Science-based, regionally-harmonized regulation of introduction of exotic natural enemies has become necessary to ensure that biological control remains a viable option for managing pests (Hunt *et al.* 2008). With the increasing costs associated with research, there is an urgent challenge to develop methods and new guidelines to fulfill these regulatory requirements. Van

Driesche & Reardon (2004) and Bigler *et al.* (2006) proposed methods for evaluating environmental impacts of biological control agents. There have been a few attempts to define what makes a good biological control agent of arthropod pests (e.g. Beddington *et al.* 1978; Kimberling 2004) and also what attributes contribute to non-target risks (Holt & Hochberg 2001; Kimberling 2004; Pearson & Callaway 2005). In addition, general principles have been proposed for predicting effectiveness of potential biological control agents. Murdoch (1994) articulated perhaps the most over-arching principle hypothesized to govern effectiveness of specialized biological control agents: agents that are able to persist at the lowest equilibrium of their prey or host should be the most effective. In addition, a number of authors have analyzed the historical record of biological control in order to identify factors or traits that are associated with success and failure (e.g. Hawkins *et al.* 1993; Hawkins & Cornell 1994; Stiling 1993, Stiling & Cornelissen 2005; Kimberling 2004). Despite these advances, there is still much room for improving in our ability to predict whether a biological control agent will succeed, fail or back fire. In this review, we provide a brief outline of some of the main issues that are the subject of the most current inquiry as an introduction to more specialized papers that follow in this session (Berkvens *et al.* 2009; Haye *et al.* 2009; Murray *et al.* 2009; Teulon *et al.* 2009).

CHARACTERIZING ATTRIBUTES OF SUCCESSFUL AGENTS.

Early summaries of biological control projects (e.g., DeBach 1974; Caltagirone 1981; DeBach & Rosen 1991; Stiling 1993) have provided insight into factors that contributed to success or failure of releases, but most do not mention what characteristics of the agents contribute to that success. Databases (e.g. Greathead & Greathead 1992) have compiled information on releases of biocontrol agents in formats that could enable the generation of statistics on successes and failures in control and these have recently been used to assess non-target impacts (e.g. Stiling & Simberloff 2000; Lynch *et al.* 2001). However, databases must be used cautiously since not all agents are thoroughly evaluated (Waage 1990). Mathematical models have been used to generate hypotheses about what attributes are associated with successful biological control agents (reviewed by Mills & Getz 1996; Murdoch *et al.* 2003; Elkinton 2008). However, relatively few empirical case studies have been used to test assumptions and predictions of these models (e.g. Hassell 1980; Godfray & Waage 1991; Murdoch *et al.* 1996). In addition, for many successful biological control projects, the general reasons for success seem relatively obvious and have been inferred empirically (Table 1).

Depending on whether an agent is intended for classical or inundative biological control the optimal state of characteristics may vary. For example, the self-perpetuation of a classical agent may require that it is able to survive extreme climate (overwinter in cold climates or aestivate in hot, dry climates) whereas agents used to overwhelm a pest in the short term (such as in glasshouse environments) may not be expected to survive extreme climates. Therefore, the optimized state may vary according to intended use. In Table 2 we have noted attributes of natural enemies predicting effectiveness that we suspect differ between classical and augmentative biological control.

Table 1. Examples of successful biological control projects and the likely reasons for their success.

Target species	Natural enemy	Likely reasons
<i>Icerya purchasi</i> Maskell	<i>Rodolia cardinalis</i> (Mulsant)	Thermal tolerance, short development time with respect to prey (DeBach & Quezada 1973)
<i>Trialeurodes vaporariorum</i> (Westwood)	<i>Encarsia formosa</i> Gahan	High dispersal ability, high searching ability, accepts all immature host stages, ease of mass rearing, (van Lenteren 1995; Hoddle <i>et al.</i> 1998)
<i>Tetranychus urticae</i> Koch	<i>Phytoseiulus persimilis</i> Athias- Henriot	Voracious feeding, high dispersal ability, high searching ability, ease of mass rearing, availability of pesticide-resistant strain (van Lenteren 1995)
<i>Phenacoccus manihoti</i> Matile-Ferrero	<i>Apoanagyrus lopezi</i> De Santis	Attack of early host instars, production of more females on young hosts, superior competitive ability, high search capacity (Neuenschwander 2001)
<i>Aonidiella aurantii</i> (Maskell)	<i>Aphytis melinus</i> DeBach	Thermal tolerance, ability to produce female offspring on relatively small scale insects (Luck 1986; Murdoch <i>et al.</i> 1996)

Table 2. Examples of attributes predicting success of agents differently for classical and augmentative biological control.

	Classical	Augmentative
Distribution in host native range/habitats	widespread	not required
Efficacy in native range	high	not required
Dispersal capability	high	short range important
Life cycle	synchronized with host	synchronization less important for inundative agents
Host specificity	high	high or low

There is no ideal set of attributes that guarantees success and some good attributes may not be associated with the best agents. For example, ease of rearing in culture is a practical aspect that has implications for early control of the target (Doutt & Debach 1964) and more recently for host range assessment. However this must be carefully considered because effective agents might be eliminated from further consideration solely because they are not easily reared (Waage 1990).

Phenotypic variability is an example of an attribute where the perceived optimum state can have both good and bad effects. In terms of climate matching, a high level of variation of a biological control agent (i.e. populations from as many areas as possible in the area of origin) is desirable to ensure that one of the

populations matches that of the area of intended introduction. However, high genetic variability may also increase the risk of non-target impacts (Phillips *et al.* 2008).

CHARACTERIZING ATTRIBUTES OF ENVIRONMENTALLY-RISKY BIOLOGICAL CONTROL AGENTS.

Whereas it is usually rather straightforward to assess whether a biological agent has become established in nature and, subsequently, has achieved an economic impact or not, it is much more problematic to assess its non-target impact on native biodiversity and ecosystems. Among the hundreds of biological control agents established worldwide, many have been found on non-target hosts or prey (Lynch *et al.*, 2001; Kimberling 2004). Kimberling (2004) tried to use this information to detect traits associated with non-target effects. She concluded that traits that could be used to predict non-target effects included sex-ratio of progeny (female biased sex ratio being associated with lower non-target effects) and the presence of native natural enemies. However, she defined “non-target effects” as evidence from the literature that an exotic agent attacks non-target native host/prey or competes with native natural enemies. But the fact that a parasitoid or a predator is found parasitizing or feeding on a non-target species does not necessarily mean that it has a significant effect on populations of the non-target species. In their extensive literature survey on the ecological effects of alien insects, Kenis *et al.* (2009) found evidence for a significant effect on native species populations in only six intentionally introduced parasitoids and two intentionally-introduced predators (Table 3). All parasitoids and predators for which a severe non-target effect has been ascertained are known to attack a high number of hosts or prey. Thus, the rather recent move towards the selection of highly specific agents in classical biological control of arthropods is a good one (Van Driesche & Reardon 2004). Interestingly, several of the parasitoids and predators that have had documented negative effects on non-target species have also been considered “successful” agents. The tachinid *Bessa remota* (Aldrich) (Diptera: Tachinidae) has even been considered as extremely successful because it is the only parasitoid, which, in the 1920s in Fiji, is suspected to have eradicated both its target host, the coconut moth, *Levuana iridescens* Bethune-Baker (Lepidoptera: Zygaenidae) and a non-target native moth, *Heriothopan dolens* Druce (Lepidoptera: Zygaenidae) (Kuris 2003). However, Kuris (2003) and Hoddle (2006) have suggested that *L. iridescens* may have survived on other islands, and also that there is little evidence *H. dolens* actually went extinct. This suggests again that traits associated with failure in control and non-target effects have to be analysed separately.

WHAT ARE WE LOOKING FOR?

There is a general consensus that no biological control agent possesses all ideal states of desirable attributes. Selection criteria based on the notion that ideal agents can be ‘built’ by combining desirable life-history attributes are likely wrong, due to the likelihood of correlated life-history tradeoffs, and a more realistic approach would be to focus on combinations of attributes that characterize real species (Waage 1990). Furthermore, characteristics associated with biological control agents that carry environmental risks are different from those associated with ‘good’ or ‘failed’ agents. As stated by Turnbull & Chant (1961), “... we must know much about the attributes that make an organism effective in limiting the abundance of others,

how this effect is modified by undesirable characters, the mechanics of interactions, and, of course, the way in which the introduced species will fit into its new environment and its chances of survival there, and the attributes that determine this."

Table 3. Alien biological control agents for which an effect has been measured on native species populations or communities (from Kenis *et al.* 2009).

Species	Mechanism involved in the effect
<u>Parasitoids</u>	
<i>Aphidius ervi</i> (Haliday) (Hym.: Braconidae)	Parasitism
<i>Bessa remota</i> (Aldrich) (Diptera: Tachinidae)	Parasitism
<i>Cales noaki</i> Howard (Hymenoptera: Aphelinidae)	Competition with native parasitoid
<i>Compsilura concinnata</i> (Meigen) (Diptera: Tachinidae)	Parasitism and competition
<i>Lisiphlebus testaceipes</i> (Cresson) (Hymenoptera: Braconidae)	Competition with native parasitoids
<i>Torymus sinensis</i> Kamijo (Hymenoptera: Torymidae)	Hybridization with native parasitoid
<u>Predators</u>	
<i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae)	Competition for food or intra-guild predation
<i>Harmonia axyridis</i> (Pallas) (Coleoptera: Coccinellidae)	Competition for food or intra-guild predation

Case studies provide a starting point for defining qualities associated with biological control agents, and some of these are in the contributions in the remainder of this section (Berkvens *et al.* 2009; Haye *et al.* 2009; Murray *et al.* 2009; Teulon *et al.* 2009). Retrospective analyses where outcomes are known may be able to provide the data for developing ideas about attributes associated with agents perceived to be effective, ineffective and risky. Carefully planned, science-based studies will provide new insights. Furthermore, the study of natural enemy complexes through population interactions (intra-guild interactions) can facilitate determining appropriate attributes (Brodeur & Boivin 2006). In other words, we must learn as much as possible about the biology of candidate biological control agents and the ecological context in which they are used.

CONCLUSIONS.

No comprehensive assessment of characteristics associated with effective, ineffective and risky biological control exists. Clearly, there is a great deal of work ahead. Mathematical models have a use to generate hypotheses and test scenarios but will need to be empirically validated. Case studies are likely to be the source of information for developing databases that can be analysed. It appears that the way forward will be to develop an approach that captures not only the characteristics associated with agents but also the ecological context in which the agents are used.

Only then will we be able to determine principles for guiding decision making on the suitability of biological control agents.

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GOOD AND BAD TRAITS OF *HARMONIA AXYRIDIS* –FROM BIOLOGICAL CONTROL TO INVASION

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ABSTRACT.

The ladybird, *Harmonia axyridis*, was introduced from Asia as a biological control agent of aphid and coccid pests and has been very successful in this respect. The coccinellid established but became invasive in surrounding regions and has been reported to have undesirable negative impacts on native aphidophagous guild members, cause nuisance problems in residential areas and also plant damage. This makes *H. axyridis* an excellent case study to identify the traits that make a predator an attractive and effective biological control agent and those that lead us to categorise it as an alien invasive species. A number of these traits can be both “good” and “bad” when viewed in either a biological control context or in an ecological context. In this paper we discuss the relative merits of traits that are commonly associated with biological control agents (voracity, prey searching efficiency, tolerance to pesticides) along with those less often considered, but that we believe are of equal importance, including overwintering potential, phenotypic adaptability and intraguild interactions with native aphidophagous species. The consequences of these traits on species diversity and the control of pest species in the introduced range will be discussed.

HARMONIA AXYRIDIS AS A BIOLOGICAL CONTROL AGENT AND ITS ESTABLISHMENT IN EUROPE.

The multicolored Asian ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is indigenous to a vast region enclosing China, Japan, Korea, Mongolia and Siberia (Brown *et al.* 2008a). Because of its high voracity and good prey searching ability, ease of rearing, wide food range and ability to colonize a wide range of habitats, the species has been used in biological control strategies in America and Europe (e.g. Brown *et al.* 2008a; Coutanceau 2006; Koch 2003). *Harmonia axyridis* was initially released as an exotic classical biological control agent against non co-evolved pest species and intended to establish and maintain self perpetuating control of pest species at the release sites. Due to its highly dispersive nature *H. axyridis* did not remain at the release sites necessitating the repeated release of *H. axyridis* and the adoption, in some areas, of an augmentation biological control approach in which establishment was no longer expected or desired. Several studies have reported the species to be a successful biological control agent in

various crops against a number of economically important aphid pests (reviewed in Hodek & Honek 1996; Koch 2003; Lucas *et al.* 2007a; Tedders & Schaefer 1994). However, in several areas where it had been augmented it established and quickly became invasive. A detailed description of the species' introduction and invasion history can be found in Koch (2003), and Koch & Galvan (2008) for North and South America and in Brown *et al.* (2008a) and Coutanceau (2006) for Europe. In some cases adverse impacts on native biodiversity, the fruit and wine industry and inhabitants of buildings in which the species overwinters en masse have been reported (Adriaens *et al.* 2008; Koch 2003). Public concern for the potential risks *H. axyridis* poses to the environment, humans and crops has raised awareness that beneficial and potentially negative properties of an exotic natural enemy need to be evaluated and acted upon before release as a biological control agent.

FUNCTIONAL TRAITS OF *H. AXYRIDIS*.

The particular biological attributes, or traits, of *H. axyridis* relate to how it functions as a predator and have evolved to ensure that it is well adapted to conditions in its native range and the ecological niche that it fills. They also determine its potential as an effective biological control agent, but also its likelihood of having negative consequences for the environment if exploited for biological control, particularly beyond its native range. Here we describe these attributes, discuss their consequences and use them to identify those that are the strongest drivers, alone or in combination, of negative consequences and are, therefore, of greatest relevance for risk assessment of biological control agents.

Foraging efficiency and prey range.

As one of the largest predatory coccinellids, *H. axyridis* consumes up to 370 aphids during larval development and up to 800 aphids in its lifetime (Koch 2003; Soares *et al.* 2001). This coupled with a well adapted search strategy makes *H. axyridis* a formidable predator (Hodek & Honek 1996; Koch 2003; Obata 1986).

Unlike many species of coccinellid, *H. axyridis* is highly polyphagous. Arthropod pest species attacked include numerous aphid species but also species in Tetranychidae, Psyllidae, Coccoidea, Chrysomelidae, Curculionidae and Lepidoptera (e.g. Hodek & Honek 1996; Koch 2003; Lucas *et al.* 2007a; Tedders & Schaefer 1994). However, polyphagy also means that *H. axyridis* has the potential to prey on non-target arthropods. Experimentation has shown that these can include species of conservation concern like the monarch butterfly (Koch *et al.* 2006) and beneficial species within the natural enemy guild such as the immature stages of predators and parasitoids as well as fungal pathogens (see Pell *et al.* 2008 and references therein).

When insect prey are scarce *H. axyridis* can exploit plant foods like pollen and nectar (Berkvens *et al.* 2008a). Pollen is a suboptimal food as larval survival and adult fecundity are both significantly reduced compared to individuals feeding on lepidopteran eggs (Berkvens *et al.* 2008a). However, it could provide a mechanism for survival in the absence of prey that most other species of coccinellid do not have (Berkvens *et al.* 2008a; Hodek & Honek 1996; unpublished data). Unlike many predatory coccinellids, *H. axyridis* can also be frugivorous in the autumn, which is believed to increase winter survival of adults (Galvan *et al.* 2008; Koch *et al.* 2004a).

Reproductive capacity.

Harmonia axyridis is multivoltine and does not require a period of diapause before reproduction. In its native and invasive range *H. axyridis* is usually bivoltine (e.g. Adriaens *et al.* 2008; Brown *et al.* 2008a; Koch 2003), but more than two generations per year can also occur, with up to four generations per year in Greece (Katsoyannos *et al.* 1997), facilitating rapid population build up. *Harmonia axyridis* females produce large numbers of offspring and can be long lived. Females can lay between 20 and 30 eggs per day, live for between 60 and 140 days with a long oviposition period, between 30 and 100 days (Agarwala *et al.* 2008; Berkvens *et al.* 2008b; Hodek & Honek 1996; Mignault *et al.* 2006; Soares *et al.* 2001), depending on the nature and abundance of prey. There are even reports of females living for 3 years and still ovipositing in their third year (Hodek & Honek 1996).

Climatic adaptability.

Development and reproduction of *H. axyridis* is optimal between 15 and 25°C but only limited below 11°C and above 35°C, and they easily tolerate subzero temperatures (Acar *et al.* 2004; LaMana & Miller 1998; Schanderl *et al.* 1985). In a number of studies in the USA and Japan, the lower lethal temperature was between -11.9°C and -19°C (Koch *et al.* 2004b; Watanabe 2002). Overwintering adult *H. axyridis* collected from Belgium, Europe had a similar degree of cold tolerance (-15 to -19°C) to those collected by Watanabe (2002) (Berkvens *et al.* unpublished data). Using the CLIMEX model Poutsma *et al.* (2008) predicted the potential geographic distribution of *H. axyridis* based on climatic variables. In addition to the countries in temperate Europe and North America in which the species has already established, the model predicted that the species could also establish in a large part of Mediterranean Europe, South America, Africa, Australia and New Zealand.

Habitat adaptability.

Harmonia axyridis is primarily categorized as an arboreal species. Favoured arboreal habitats in Belgium, for example, include lime (*Tilia* sp.), maple (*Acer* sp.), willow (*Salix* sp.), oak (*Quercus* sp.), birch (*Betula* sp.), pine (*Pinus* sp.) and hawthorn (*Crataegus* sp.), but nettle (*Urtica dioica* L.(Urticales: Urticaceae)) and reed (*Phragmites australis* Steud (Cyperales: Poaceae)) are also frequently visited (Adriaens *et al.* 2008). However, unlike many coccinellids that are habitat specialists, *H. axyridis* can be considered eurytopic as it is found in a vast range of semi-natural, agricultural and even urban habitats (e.g. Adriaens *et al.* 2008; Brown *et al.* 2008b; Lucas *et al.* 2007b).

Dispersal capacity.

Harmonia axyridis has a high dispersal capacity. In North America it spread over an area of nearly 150 000 km² during the two years after it initially established in 1988 in south-eastern Louisiana (Teddars & Schaeffer 1994). In Europe it has established in 13 European countries in the nine years after it was first recorded in the wild in 1999 (Brown *et al.* 2008a). High dispersal rates for individual *H. axyridis*

adults are evident in the long migratory flights to their hibernation sites (e.g. Brown *et al.* 2008a; Hodek & Honek 1996).

Polymorphism and phenotypic plasticity.

Harmonia axyridis is highly polymorphic, with more than 100 recorded melanic and non-melanic elytral patterns (Soares *et al.* 2003). Recent studies indicate there is variation among the colour morphs of *H. axyridis* for different traits (e.g. food conversion, developmental time, fecundity, body size and weight, etc.), offering particular morphs greater fitness than others in specific habitats or at specific times (Berkvens *et al.* 2008 ab; Serpa *et al.* 2003; Soares *et al.* 2001, 2005;). The most prominent colour morph varies from region to region (Hodek & Honek 1996; Koch 2003), suggesting that some phenotypes are indeed favourably selected in different ecosystems (Soares *et al.* 2008). Additionally, colouration and maculation of the species can be influenced by temperature during development (Berkvens *et al.* 2008b). Thermal melanism may speed up development and allow adults to remain active at lower temperatures, thereby increasing their foraging capacity before overwintering and their competitive advantage over other predatory species lacking this strategy (Majerus 1994).

Natural defences.

Harmonia axyridis has several natural enemies including pathogens, parasitoids, nematodes, parasitic mites and certain bird species (for a full review see Kenis *et al.* 2008; Koch 2003). However, there are only a few recorded observations of these natural enemies effectively attacking or parasitizing *H. axyridis* in its invaded range. Emergence rates of the braconid *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) were near to zero when attacking field-collected *H. axyridis* adults in the USA (Hoogendoorn & Heimpel 2002) and Canada (Firlej *et al.* 2005) and varied between 0 and 10 % when parasitizing European field populations (Berkvens *et al.* unpublished). Parasitism by the tachinid fly *Strongygaster triangulifera* (Loew) (Diptera: Tachinidae) in North America varied between 4.8 and 15.4% (Katsoyannos & Aliniasee 1998). *Harmonia axyridis* is less susceptible to the nematode *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) and the fungus *Beauveria bassiana* (Balsamo) (Deuteromycotina: Hyphomycetes) than other coccinellids tested (Roy *et al.* 2008; Shapiro-Ilan & Cottrell 2005). Interestingly, *H. axyridis* has a tolerance to certain insecticides, insect transgenic crops and fungicides similar to or higher than other natural enemies of aphids (for a full review see Koch 2003).

As with other coccinellid species, when attacked, *H. axyridis* has several effective defence strategies including aposematic colouration, reflex bleeding of alkaloid laden secretions, chemical defence of eggs, thanatosis and dropping behaviour (e.g. Koch 2003; Ware *et al.* 2008). Moreover, third and fourth instars have spines that provide physical protection from intraguild and higher-order predation (Soares *et al.* 2008; Ware *et al.* 2008).

BENEFICIAL AND ADVERSE CONSEQUENCES.

When we consider the functional traits of *H. axyridis*, they describe an organism that is extremely likely to establish in an ecosystem in which it is introduced. Its polyphagy allows it to persist by exploiting alternative food resources. It is also large, voracious, multivoltine and has a high reproductive rate. As such it has potential as a classical biological control agent where establishment as well as efficacy in a target habitat is a prerequisite. For augmentation biological control establishment is not desired. However, polyphagy and high reproductive capacity facilitate ease of mass rearing and ensure a large market potential against a variety of pest species. Its ability to track pest aphid population growth and tolerance for a variety of pesticides also facilitate its use in integrated pest management programs. These aspects contributed to the perception that *H. axyridis* also had utility as a commercially viable augmentative biological control agent and was how it was ultimately used most extensively.

The very same traits that can be favourable, independently, in a biological control agent also strengthen the invasive character of a species if they occur in combination, as they do with *H. axyridis*. Alongside its other traits, *H. axyridis* is also polymorphic, dispersive, eurytopic and thus adapted to exploit a wide climatic and habitat range and well able to disperse between habitats giving it the potential to go beyond establishment in a target habitat to being an exotic invader with potential for widespread negative ecological impacts in a wide range of non-target native ecosystems. Where other biological control agents may exhibit one or two traits that make them effective in pest regulation but not a risk beyond the target habitat, *H. axyridis*, has a wide range of traits that independently may be useful in biological control, but together have significant negative consequences.

Furthermore, many traits of *H. axyridis* are exhibited to an extreme degree. For example, most indigenous coccinellids in Northwest Europe only have one generation per year (e.g. Majerus & Kerans 1989) and are less fecund than *H. axyridis* (Hodek & Honek 1996), offering *H. axyridis* a higher potential population growth than the native species (Brown *et al.* 2007a) and thus a competitive advantage.

Polyphagy in *H. axyridis* does not just extend to a number of prey species within one order but to species across many orders and even beyond arthropods to plants. Direct feeding on fruit and contamination in harvested wine grapes may make *H. axyridis* an organism of plant health concern (Galvan *et al.* 2008; Koch *et al.* 2004). A potentially stronger force structuring communities as a whole is intraguild predation which occurs when two species in the same guild also engage in a trophic interaction with each other (Polis *et al.* 1989). *Harmonia axyridis* is larger and better defended than the majority of other guild members (including predators, parasitoids and pathogens) and so the outcome is usually asymmetrical in favour of *H. axyridis* (Lucas *et al.* 2007a; Pell *et al.* 2008 and references therein). Petri dish experiments represent a 'worst case scenario' and the outcome may be different at more realistic scales and degrees of environmental complexity, where intraguild prey can escape or avoid contact. Natural enemies in the same guild can also facilitate each others activity. For example, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) increases transmission and dispersal of the aphid specific fungal pathogen *Pandora*

neoaphidis (Remaudière and Hennebert) Humber (Zygomycota: Entomophthorales) (Pell *et al.* 1997; Roy *et al.* 1998; 2001) and *H. axyridis* has recently been shown to increase fungal transmission to a similar extent (Wells *et al.* 2008). In general, however, *H. axyridis* has been associated with declines in population densities of some native coccinellids in its invasive range suggesting that it can have a negative impact on the diversity of other guild members (e.g. Adriaens *et al.* 2008; Michaud 2002).

Intraguild interactions do not always require the mortality of the shared prey (i.e. the target pest) and can, therefore, result in a reduction in predation pressure on the target pest population – i.e. an overall reduction in biological control (Rosenheim *et al.* 1995). In single generation experiments the addition of *H. axyridis* to an aphid enemy guild did not reduce target prey suppression even when intraguild predation occurred (Aquilino *et al.* 2005; Cardinale *et al.* 2003; Ferguson and Stiling 1996; Gardiner & Landis 2006; Snyder *et al.* 2004; Snyder & Ives 2003). However, over a larger timescale *H. axyridis* could reduce the stability of the ecosystem service of pest suppression that is provided by a diversity of natural enemies buffering for each others activity in a changing environment (Gardiner & Landis 2006; Pell *et al.* 2008; Snyder & Ives 2003)

Additionally, overwintering of *H. axyridis* adults in aggregations in buildings can cause nuisance problems for humans inhabiting or working in these buildings because its aggregations can be much larger than those of native coccinellids. There have also been reports of people developing allergic reactions to the coccinellid and even reports of *H. axyridis* biting humans (Huelsman 2002; Yarrow *et al.* 1999).

CONCLUSIONS.

The high pest suppression potential and polyphagy of *H. axyridis* make it an attractive candidate for augmentative biological control of a number of aphid and coccid pests. In addition, the insect's establishment potential and dispersal ability would be assets for classical biological control. However, use of the predator in augmentative or classical biological control becomes far less appropriate if the potential environmental risks are considered. The very same traits underlying its value for pest suppression, contribute to the risk of unwanted effects, including its undesired establishment in non-target habitats (following augmentative releases) coupled with direct and indirect impacts on the aphidophagous guild or other non-target species and its status as an occasional plant pest. The use of a non-indigenous species for biological pest control is especially risky if the natural enemy is a generalist (Howarth 2001 and the references therein). *Harmonia axyridis* is an excellent case study to demonstrate that, like other crop protection strategies, biological control is not risk free and that risk analysis is the proper process to weigh beneficial versus potential adverse impacts of biological control agents, particularly if these are not indigenous. A risk assessment by van Lenteren *et al.* (2008) concluded that *H. axyridis* should never have been released as a biological control agent in Europe, given that evidence indicating the potential risk was available at the time of its commercialization in 1995. Although some of the potential adverse effects, for which there have been strong indications in numerous studies, may not have been realized in the field at present, few workers will challenge the conclusions of this risk assessment. Biological control can offer a safe alternative option for pest control,

particularly if it is integrated with other control options. However, it is important that our expectations of biological control are realistic and that risk assessments take into account all the functional traits of the organism to be used and their implications for the community into which the organism is released.

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PREDICTIVE VALUE OF BIOLOGICAL CONTROL AGENT'S ATTRIBUTES FOR INTRODUCTION: *PERISTENUS DIGONEUTIS* AS A CASE STUDY

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ABSTRACT.

When searching for promising biological control agents, generations of researchers have tried to find a sure way of predicting beforehand which biological control agents will lead to economic pest suppression and which will fail. Although many approaches have been proposed for characterizing 'good' biological control agents, most of these have only resulted in general guidelines with limited predictive value. To facilitate the evaluation of the potential of biological control agents prior to release, a variety of desirable attributes have been suggested. Among others these include, a high level of host specificity, a good searching capacity, a shorter generation time, a positive response to increasing host densities, a good temporal synchronisation of host and agent, and a good dispersal ability. However, the variable outcome of past biological control projects urges the need for reviewing the predictive value of these attributes. Particularly reviewing biological control agents that have been proven to be successful in the past can help to identify those general characteristics that lead to economic pest suppression. An example for a successful new association biological control agent is the European *Peristenus digoneutis*, which has significantly reduced native *Lygus lineolaris* populations after its establishment in the eastern United States in the 1980s. In the present study we review current knowledge on *P. digoneutis* and try to relate certain biological attributes accountable for the success or failure of release programmes in the past. *Peristenus digoneutis* as a case study in the review of desirable attributes of biological control agents suggests that a broad understanding of the ecology and interactions of a biological control agent and its target pests in the area of origin is the key factor for a reliable evaluation of the potential success of a biological control project.

INTRODUCTION.

Since the early days of biological control, researchers have tried to predict, prior to release, which biological control agents will succeed and which will fail in sufficiently suppressing a pest. However, interactions between the agent, its target and the new environment are complex and often very subtle and therefore exceedingly difficult to predict. Typically a 'good' biological control agent should be biologically, physiologically and ecologically well adapted to the target species and the environmental conditions it will be released in. Its effectiveness is determined by a broad range of factors, all contributing to the ultimate goal of causing a significant impact on the pest population, without posing an unacceptable risk to non-target

species. To help assess the potential of biological control agents prior to release, a variety of desirable attributes have been suggested by various authors (Coppel & Mertins 1977; Kimberling 2004; Mills 1990; Murdoch *et al.* 2003), but it has also been realized that in each agent 'good' and 'bad' attributes are combined due to the trade-offs in the evolution of its life history traits (Mills 2005). Consequently, the pros and cons of biological control agents' attributes have to be evaluated case by case and only a few general principles for the selection of the most promising biological control agents exist at present.

One way to improve our understanding of what makes a 'good' agent is by combining the knowledge gained from past biological control programs with a better documentation of life history characteristics (Kimberling 2004). An example of a successful biological control agent is the European *Peristenus digoneutis* Loan (Hymenoptera: Braconidae), which was introduced into North America to reduce *Lygus lineolaris* (Palisot) (Hemiptera: Miridae) populations. Native North American *Lygus* parasitoids in the genus *Peristenus* Foerster and *Leiophron* Nees (Hymenoptera: Braconidae) were considered ineffective, as nymphs of the first *Lygus* generation were primarily attacked, and subsequent *Lygus* generations were usually not significantly parasitized (Day 1987; Braun *et al.* 2001). Thus, surveys for exotic parasitoids were extended to the Palaearctic where three promising European *Peristenus* species were discovered, including *P. digoneutis*, a parasitoid of the European plant bug *Lygus rugulipennis* (Poppius) (Heteroptera: Miridae) (Loan & Bilewicz-Pawinska 1973). Following a new association approach, the later was first released into alfalfa fields in New Jersey in 1973 (Coulson, 1987). In the following, collections of *P. digoneutis* in Europe with subsequent releases in various regions of North America continued sporadically until today. Whereas releases in Western Canada were without apparent success, *P. digoneutis* was successfully established in New Jersey in the mid 1980s (Day *et al.* 1990) and just recently in California (Pickett *et al.* 2007). After its establishment and spread throughout the northeastern US, *P. digoneutis* reached high levels of parasitism and decreased *Lygus* densities in alfalfa by 75% (Day, 1996).

Traditionally, when searching for effective biological control agents, certain promising attributes are obvious prior to introduction; among others these include a high level of host specificity, a good searching capacity, a shorter generation time, a positive response to increasing host densities, a good temporal synchronisation of host and agent, and good dispersal ability. In this study, we aimed to review the research on *P. digoneutis* over the last 35 years, trying to answer the question, which parasitoid attributes or other factors may have contributed to success and failure of release programs, retrospectively.

MATERIALS AND METHODS.

For reviewing the current knowledge of the attributes associated with *P. digoneutis*, studies published since its first description in 1973 were examined using CAB Abstracts and Google Scholar. The database generated 52 studies, covering a very broad range of topics, including studies on impact on the target, host specificity, competition, distribution, dispersal, rearing, and molecular detection methods.

RESULTS.

Ecological Characteristics and Compatibility.

Foreign exploration for parasitoids of *Lygus* spp. within the last 35 years have shown that *P. digoneutis* is widely distributed within central Europe, including countries like Poland, Austria, France, Spain, Germany, and Switzerland (Coutinot & Hoelmer 1999; Gariepy *et al.* 2008c; Haye *et al.* 2005b). Its northern distribution is assumed to be limited to Denmark or southern Sweden. Recent studies state its absence from northern Sweden and Finland, where *Lygus* species can only complete a single generation due to a shorter vegetation period (Rämert *et al.* 2005; Varis & van Achterberg 2001).

Like its European host *L. rugulipennis*, *P. digoneutis* does not seem to be restricted to a particular habitat and has been recorded on many host plants of different families (e.g. alfalfa, clover, barley, rye, oats, and potatoes). However, in Poland the highest parasitism levels by *Peristenus* species were generally observed in long-term habitats, such as alfalfa fields (Bilewicz-Pawinska 1977).

Whether, *P. digoneutis* could actually survive winter conditions in the Canadian Prairie Provinces is not known. The supercooling point of *P. digoneutis* (-22.5°C to -26.8°C) is similar to those measured for native North American *Peristenus* species (SCP = -22.44 °C ± 1.97) such as *P. mellipes* Cresson and *P. pseudopallipes* (Loan) (Hymenoptera: Braconidae) (Haye 2004; Panneton *et al.* 1995). In terms of overwintering, the freeze-intolerant *P. digoneutis* is able to acclimatize to changing temperatures with an increase/decrease of its supercooling point. However, it has been demonstrated that long-term exposure to low temperatures above the supercooling point can cause high mortality in diapausing *P. digoneutis* (Haye 2004).

Temporal Synchronisation.

High parasitism levels of *L. lineolaris* in the area of release in eastern North America (Day 1996) suggest that the life cycle of *P. digoneutis* is well synchronized with the phenology of its target host. This is likely because of the exceptionally similar phenology of the ecologically equivalent hosts, *L. rugulipennis* in Europe and *L. lineolaris* in eastern North America. Both species usually complete two generations per year and in climatically suitable regions, they can have a partial third generation (Afscharpour 1960; Day *et al.* 1998). In general, *P. digoneutis* is regarded as a bivoltine species having one generation in spring and one in mid summer, but it was also observed that a small proportion (~13%) of the first generation can enter into an obligatory diapause without forming a second generation in the same year (Haye 2004). Field data from southern Ontario in Canada, suggest that, like its target, *P. digoneutis* can also have a partial third generation (Broadbent, unpublished data).

Density Responsiveness and Searching Capacity.

The similarity in the length of development time of *P. digoneutis* (35 days from egg to adult, Bilewicz-Pawinska 1982) and its host, *L. lineolaris* (ca. 38 days, Khattat & Stewart 1977) does not allow any numerical response of the parasitoid to

increased *Lygus* densities. Long-term field data, demonstrating that high parasitism levels were associated with higher *Lygus* densities (Day 1996), suggest that *P. digoneutis* shows a functional response by increasing its attacks against increasingly numerous *Lygus* hosts. In Europe, clover, alfalfa, and chamomile seem to be much more attractive to *Lygus* than other host plants. Thus, it has been assumed that high parasitism on these host plants might be partially influenced by higher *Lygus* densities (Gariépy *et al.* 2008b). Whether *P. digoneutis* has the ability to find its host at very low densities remains unclear. The host-searching behaviour of *P. digoneutis* and any chemical cues used for host and habitat location are still unknown, and further investigation is required.

Reproductive Attributes.

The solitary, synovigenic *P. digoneutis* lays single eggs inside the nymphal stages of its host, and the average potential lifetime fecundity is 385 ± 35 SE eggs (Haye *et al.*, 2005a), which seems fairly high to begin with. However, the lifetime fecundity of *P. digoneutis* needs to be re-evaluated if compared to the lifetime fecundity of another *Lygus* parasitoid, *Peristenus relictus*, which shares the same ecological niche, but has a 50% higher mean potential lifetime fecundity reaching over 782 ± 65 SE eggs. In culture, Whistlecraft *et al.* (2000) observed a mostly male biased sex ratio with (15-40% females, depending on the rearing method).

Dispersal Capacity.

Since its first recovery at the original release site in New Jersey in 1984, *P. digoneutis* has naturally dispersed 900 km northeast wards into Canada, where it is nowadays present in plant bug populations in Quebec, southern and eastern Ontario, and Nova Scotia (Gariépy *et al.* 2008a; Goulet & Mason 2006). Furthermore, it moved about 600 km westwards as far as Ohio (USA) but only 160 km southwards, which is probably caused by a lower heat tolerance of *P. digoneutis* (Day *et al.* 2008).

Host Specificity.

According to Stiling (2004), *P. digoneutis* has to be categorized as a generalist, as it has recently been recorded from a total of 10 genera, including host records from Europe (Gariépy *et al.* 2008c; Haye *et al.* 2005b) and North America (Day 1999). However, in the later studies it was also demonstrated that *P. digoneutis* primarily attacks *Lygus* species, and the impact on non-targets was low due to the very low level of parasitism in non-targets in the area of origin and the area of release. Thus, *P. digoneutis* should be categorized as an oligophagous species.

Antagonistic Interactions.

In eastern North America hyperparasitism levels by *Mesochorus curvulus* Thomson (Hymenoptera: Ichneumonidae) in *P. digoneutis* and other primary parasitoids were generally low (1-11%), indicating that *M. curvulus* does not have a strong negative effect on the biological control agent (Day 2002). In Saskatchewan (Canada) 30% of native *Peristenus* species attacking *Lygus* were hyperparasitized by *M. curvulus* and a second unidentified *Mesochorus* species (Ashfaq *et al.* 2005). In Europe, *Mesochorus* sp. has been reported to attack *Peristenus* species in *L.*

rugulipennis populations across Poland (Bilewicz-Pawinska 1975), but specimens were not identified to species level. In Northern Germany, apparently the same species attacking *Peristenus* in North America, *M. curvulus*, was reared from *Lygus* species, but parasitism levels were generally low (<10%) (Haye 2004). Direct competition between the native *P. mellipes* (syn. *P. pallipes*) and *P. digoneutis* is regarded a minor factor because *P. mellipes* usually occurs earlier than *P. digoneutis* during the first *Lygus* generation and is absent in the second (Day 2005). Furthermore, Lachance *et al.* (2001) demonstrated that *P. digoneutis* is a better in-host competitor than the native *Leiophron lygivorius* (Loan) (Hymenoptera: Braconidae). Several other *Lygus* parasitoids native to North America have just recently been described (Goulet & Mason 2006), but potential competition with *P. digoneutis* has yet to be documented.

Culturability.

To facilitate the breeding of control agents for establishment and distribution, mass rearing techniques are essential. For both, the *Lygus* host and *P. digoneutis*, effective rearing methods are available, allowing the production of 1,500 parasitoids per month (Whistlecraft *et al.* 2000).

Systematic Relationships.

The species diversity of *Peristenus* attacking *Lygus* species in Europe was characterized at an early stage of biological control efforts (Loan & Bilewicz-Pawinska 1973). Thus, the failure of the biological control program, due to inadequate taxonomy knowledge, was prevented. *Peristenus* species associated with North American *Lygus* species were just recently reviewed (Goulet & Mason 2006), and demonstrated that interactions between native parasitoids and *Lygus* species are probably much more complex than previously assumed.

Maximum Parasitism.

In the area of origin, average parasitism by *P. digoneutis* is about 35% (Garipey *et al.* 2008c), but maximum parasitism was occasionally found to be higher than 70% (White 2002).

DISCUSSION.

Within the last 35 years a broad knowledge on the attributes of *P. digoneutis* has been accumulated in pre- and post-release studies in Europe and North America. However, the complexity of the *Lygus-Peristenus* system makes it still difficult to analyze, which key attributes caused the eventual success *P. digoneutis*. To improve our general understanding of which attributes make a 'good' biological control agent, Kimberling (2004) conducted a meta-analysis of historical data from biological control projects in the United States. Accordingly, certain attributes of biological control agents were significantly associated with the success of biological control programs. Particularly, non-polyphagous endoparasitoids with female dominated sex ratios, multiple generations (with respect to their hosts) and ovipositions sites within or on hosts would have the highest probability of success. With regard to these six attributes, our review on *P. digoneutis* shows that the agent

exhibits a set of 4 ‘good’ attributes, which are also accompanied by two less desirable attributes (Table 1). The analysis of additional descriptive variables furthermore suggested that natural enemy introductions are likely more successful when no native enemies (competitors) are present in the area of release. However, the main reason for releasing *P. digoneutis* into North America was that the present native *Lygus* parasitoids were considered ineffective and that it was assumed that adding a new natural enemy would actually have a positive synergistic effect on reducing the pest. Post-release studies indeed showed that competition was probably a minor factor, because *P. digoneutis* did not replace the native *P. mellipes* and overall parasitism was significantly increased (Day 2005). Another important criterion in evaluating a parasitoid, is the maximum parasitism level it can cause within its area of origin. Hawkins & Cornell (1994) stated that empirical data suggest an association between maximum parasitism of parasitoids in their area of origin and their success in biological control. In particular, parasitoids causing maximum parasitism levels of above 32% are more likely to be successful in biological control programs (Table 1). After its release in eastern North America, *P. digoneutis* caused similar maximum parasitism levels (60%) as observed in Europe (ca. 70%) and reduced *Lygus* populations by 65% (Day 2005). Thus, *P. digoneutis* can be seen as another example supporting this hypothesis.

Table 1. Attributes of *P. digoneutis* and their association to probability of success in biological control projects according to empirical data by Kimberling (2004) and Hawkins & Cornell (1994)* (+ = probability for success higher; - = probability for success lower).

Attributes	Positive association with success, if attribute is	Attribute of <i>P. digoneutis</i>	Evaluation of <i>P. digoneutis</i> ' attributes
Parasitoid/predator	parasitoid	parasitoid	+
Host specificity	not polyphagous	oligophagous	+
Sex ratio	female biased	male biased	-
Generations/year with respect to the host	multiple	identical (2-3)	-
Oviposition site	in host	in host	+
Feeding site	internal (host)	internal (host)	+
Maximum parasitism in area of origin *	> 32%	ca. 70%	+

Due to concerns regarding the environmental safety of biological control, host specificity has become one of the most important criteria when evaluating biological control agents. Empirical data by Kimberling (2004) suggest that the proportions of non-target effects in past biological control programs were lower when the biological control agents were monophagous species with female dominated sex ratios, lower host mortality per individual and moderate dispersal ability. Furthermore, the proportion of non-target effects was lower when native enemies were not present.

Peristenus digoneutis, however, has a male biased sex ratio, causes a mortality of more than 100 host individuals per agent, has a good dispersal ability and in

addition, plenty of native *Lygus* parasitoids are present in North America (Table 2). In other words, the only ‘good’ attribute associated with a low risk of non-target effects, is that *P. digoneutis* is not polyphagous. On the other hand ecological host range studies in Europe and North America showed that the impact on non-targets is extremely low (Day 1999; Haye *et al.* 2005b) and contradict the results of this analysis. The ability of *P. digoneutis* to disperse into a wide variety of *Lygus* habitats would be associated with a higher probability of non-target effects, but particularly this attribute has contributed to preventing any host refuges, resulting in an area wide suppression of *Lygus* plant bug populations. As a positive side effect, it is further assumed that *Lygus* damage in apple orchards has already decreased due to the rapid spread of *P. digoneutis* (Day *et al.* 2003).

Table 2. Attributes of *P. digoneutis* and their association to probability of non-target effects in biological control projects according to empirical data by Kimberling (2004)* (+ probability for non-target effects lower; - = probability for non-target effects higher).

Attributes	Negative association with non-target effects, if attribute is	Attribute of <i>P. digoneutis</i>	Evaluation of <i>P. digoneutis</i> ’ attribute
Host specificity	not polyphagous	oligophagous	+
Sex ratio	female biased	male biased	-
Host mortality/agent	<100	>100	-
Dispersal ability	moderate	good	-
Native parasitoids present in release area	Not present	present	-

Apart from the attributes mentioned in Tables 1 and 2, a variety of additional attributes have been suggested to characterize a ‘good’ biological control agent. These include a good searching efficiency, a positive response to higher host densities, the absence of any antagonistic interactions (e.g. hyperparasitism, cleptoparasitism), a good temporal synchronisation of the agent with its host and a good ecological compatibility (e.g. Coppel & Mertins 1977; Mills 1990; Murdoch *et al.* 2003). As mentioned earlier, it is still not known if *P. digoneutis* has the ability to find its host at very low densities. However, long-term post-release studies suggest that *P. digoneutis* shows a positive functional response to increasing *Lygus* densities (Day 1996). The high level of parasitism and the significant reduction of *Lygus* populations further suggest that the life cycle of *P. digoneutis* is very well synchronized with its new association host, *L. lineolaris*, in eastern North America. The absence of any antagonistic interactions with other members of the natural enemy complex has frequently been pointed out as an important criterion for the selection of natural enemies (Mills 1990), but on the other hand hyperparasitism is also known to promote stability in host-parasitoid model systems (Luck *et al.* 1981). Post-release studies by Day (2002) indicate that the presence of the hyperparasitoid *M. curvulus* had at least no negative impact on *P. digoneutis* populations in eastern North America. Whether hyperparasitism levels of 30% in native *Peristenus* species and the presence of a second undescribed *Mesochorus* species in the Saskatoon

area (Ashfaq *et al.* 2005) may have contributed to the failure of releases in Western Canada remains unclear as long as the taxonomy, biology and host associations of *Mesochorus* species attacking *Peristenus* species is unclear.

Like most successful biological control programs, understanding the ecology of both, the host and the parasitoid, has been essential for the success of *P. digoneutis*. *Lygus* plant bugs are typical *r*-type species, feeding on pioneer weeds in disturbed environments. As soon as the food plant quality has deteriorated, *Lygus* adults are tempted to migrate to new sites and build up new populations. Thus, parasitoids are forced to constantly follow their hosts. One of the most important observations was made in Poland (Bilewicz-Pawinska 1977), where the highest level of *Lygus* parasitism was found in long-term habitats that were largely undisturbed and provided the opportunity for the parasitoids to complete the entire annual life cycle in the same location with the assurance of suitable *Lygus* hosts for two subsequent generations. The decision to release *P. digoneutis* first in perennial alfalfa fields rather than in annual crops was probably a more important factor for its establishment rather than any of its attributes. Well-managed alfalfa stands can last an average of five years with some remaining productive for 7-10 years. This rather unusual, perennial *Lygus* habitat provides plant bugs with a continuous supply of high quality food preventing *Lygus* from migrating. Even more importantly, this constant supply of hosts over time also enables the build-up of large parasitoid populations. Furthermore, during releases in the 1970s and 1980s, only a small proportion of alfalfa fields were treated with insecticides (5-20%) due to the successful applications of biological control against other insect pests in alfalfa (Day 1987). The impact of currently used insecticides in alfalfa on the beneficial *P. digoneutis* is not known, but a lack of pesticide use has been considered when selecting release sites in California (Hoelmer & Kirk 2005).

In contrast to the control efforts in eastern North America, release programs in western Canada failed. It has been assumed that past introductions were unsuccessful due to inadequate numbers of adults released, poorly adapted populations or a male-biased sex ratio (Broadbent *et al.* 2002). Recent studies indicate that it is not any specific attribute of *P. digoneutis* but more likely ecological characteristics of the agent in the area of origin that may explain why until today it did not establish in area around Saskatoon (SK, Canada). In this area, it is assumed that *L. lineolaris* has a variable number of generations and that in some years it can be univoltine (Braun *et al.*, 2001). Furthermore, the most frequently recorded *Lygus* parasitoid is the native, univoltine *Peristenus otaniae* Goulet, which parasitizes only nymphs of the first generation even when an occasional second *Lygus* generation occurs (Goulet & Mason 2006). In northern Sweden and Finland, with similar climatic conditions to Saskatchewan, *Lygus* completes one generation per year and only occasionally a second. The dominant parasitoids are the univoltine *P. pallipes* and *P. varisae*, respectively (Rämert *et al.* 2005; Varis & van Achterberg 2001), whereas bivoltine parasitoids like *P. digoneutis* are absent. This strongly implies that primarily bivoltine parasitoids may not be able to survive in areas, where two consecutive *Lygus* generations are not guaranteed. At the time of *P. digoneutis* releases in Saskatchewan, the northern distribution of *P. digoneutis* in Europe (Rämert *et al.* 2005; Varis & van Achterberg, 2001) as well as the phenology of *Lygus* species and their native parasitoids in Western Canada (Braun *et al.* 2001; Goulet & Mason 2006) was poorly understood. This evidence highlights that detailed knowledge on the

ecological requirements of the agent and target in the area of origin and introduction is essential to prevent the failure of biological control programs.

Peristenus digoneutis as a case study for reviewing the value of desirable attributes of biological control agents supports once again the view point of Legner & Bellows (1999) and Mills (2005), considering a more holistic approach for selecting 'good' biological control agents. Firstly, the most complete knowledge on the ecology and interactions of a biological control agent and its target pests in the area of origin is still fundamental for a reliable evaluation of the potential success of a biological control project. Particularly, information from the area where the biological control agent reaches its limit in distribution can be of high value to understand its ecological requirements. Secondly, even if empirical data suggests an association between maximum parasitism rates of parasitoids and their success in biological control, it is worthwhile to evaluate the meaning of high parasitism in terms of impact on population mortality of the targeted pest. Comparative life-table studies in the area of origin and introduction of the pest can be informative to evaluate the significance of potential biological control agents on population dynamics of the target pest. Thirdly, assuming key attributes of biological control agents have not been overlooked during the long history of biological control, new tools should be used to improve the accuracy of data on biological and ecological characteristics of biological control agents. Climate matching software (e.g. CLIMEXTM), which is already used to predict the potential distribution of introduced pests (e.g. Olfert *et al.* 2004), can also be applied to predict the potential distribution of biological control agents in the area of introduction and the identification of the most promising collection area for foreign exploration (Hoelmer & Kirk 2005). Furthermore, the impact of pesticides used in the area of release is often poorly understood, but guidelines for testing the side-effects of pesticides on natural enemies (Candolfi *et al.* 2000) have been developed by the IOBC working group "Pesticides and beneficial organisms" for use in the registration of pesticides. These can also be used prior to releases for testing the impact on classical biological control agents to select species that are less susceptible. The use of diagnostic molecular markers in the area of biological control is also a powerful new tool, which is already applied where traditional methods reach their limits (e.g. the identification of morphologically similar parasitoid species, cryptic species and strains). The use of molecular diagnostics in biological control is still at the beginning, but the broad fields of application (Garipey *et al.* 2007) give biological control workers the possibility to branch out in new directions in the future. Lastly, meta-analysis' of historical data from biological control projects (e.g. Kimberling 2004; Stiling 2004) are certainly a helpful contribution for developing guidelines for intentional introductions and predicting the likelihood of non-target effects. However, the present example also demonstrates that evaluating biological control agents using a simplified ranking system can be of limited predictive value and may result in the exclusion of potentially 'good' candidates.

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COMPARING THE BEHAVIOURAL STRATEGIES OF TWO PARASITOID WASPS: IS AGGRESSIVE RESOURCE DEFENDING GOOD FOR BIOLOGICAL CONTROL?

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ABSTRACT.

Paropsis charybdis is a pest of *Eucalyptus* in New Zealand and is controlled by two egg parasitoids (Hymenoptera: Pteromalidae). The first, *Enoggera nassau*, was intentionally introduced in 1987, while the second, *Neopolycystus insectifurax*, was self-introduced and discovered in 2001. Both are known to be oligophagous and can achieve almost 100% parasitism of *P. charybdis* in the laboratory. A review of surveys conducted between 2002 and 2005 suggested that *N. insectifurax* does not parasitise *P. charybdis* as successfully as *E. nassau* in the field. Preliminary comparisons of the two species in the laboratory indicate that they have quite different oviposition strategies. When competing directly with *E. nassau*, *N. insectifurax* spends much of its time aggressively defending the patch of host eggs. As *E. nassau* is almost always chased away, it spends much less time in contact with the host eggs, but during almost all egg contact it displays behaviours related to oviposition. The strategy of *N. insectifurax* results in a higher number of ovipositions in the laboratory when in direct competition with *E. nassau*. We discuss the trade off between time spent in aggressive defence of an existing patch versus time spent on actual oviposition, and the possibility that the aggressive behaviour of *N. insectifurax* reduces its efficiency as a biological control agent of *P. charybdis*.

INTRODUCTION.

The Australian beetle *Paropsis charybdis* Stål (Coleoptera: Chrysomelidae) was first discovered in New Zealand in 1916 and remains the country's most serious *Eucalyptus* (Myrtaceae) defoliator (Clark 1930; Withers 2001). In 1987 *Enoggera nassau* (Girault) (Hymenoptera: Pteromalidae) was introduced and released as a biological control agent of the pest (Bain & Kay 1989). The egg parasitoid established quickly and spread throughout the beetle's range, providing effective control in many parts of the country (Kay 1990). In 2001 a second parasitoid *Neopolycystus insectifurax* Girault (Hymenoptera: Pteromalidae) was discovered parasitising *P. charybdis* eggs in the Bay of Plenty region of the North Island (Berry 2003) and has since extended its range to the South Island (Murray *et al.* 2008).

Following the detection of *N. insectifurax* its seasonal abundance and that of *E. nassau* have been monitored in the field, although only one year of data has previously been presented (Jones & Withers 2003). Preliminary analysis of these data and general observations suggest that *N. insectifurax* parasitises fewer *P. charybdis* than *E. nassau* in the field.

In the laboratory, groups of *E. nassau* will parasitise 100% of *P. charybdis* eggs provided over 24 h, however groups of *N. insectifurax* require up to 3 days to consistently achieve similar rates (Murray unpub. data). Observations of both *E. nassau* and *N. insectifurax* (Mansfield unpub. data) in the laboratory found that both species will engage in physical combat with their conspecifics and provided the first clues that these two apparently similar parasitoids have very different oviposition strategies.

Here we present three consecutive years of parasitism data from eucalypt plantations in New Zealand and some preliminary observations on the behaviour of *E. nassau* and *N. insectifurax* when subject to interspecific competition for hosts.

MATERIALS AND METHODS.

Field Parasitism.

Parasitism by *E. nassau* and *N. insectifurax* was monitored in the Bay of Plenty and Taupo regions of New Zealand's North Island between November and March each summer from 2002 to 2005. Two sites (*Rotoiti*, 38°03'S, 176°27'E; *Murphy's*, 38°20'S, 176°25'E) were monitored each year while *Rotomahana* (38°14'S, 176°26'E) was monitored only in 2002-2003 (Jones & Withers 2003) and *Kapenga* (38°13'S, 176°13'E) only in 2004-2005 (Mansfield & Withers unpub. data). At each site approximately 10 trees bearing flushing foliage were randomly selected and adult foliage was either cut from the tree or inspected from the ground every 7-10 days for batches of *P. charybdis* eggs. All egg batches were returned to the laboratory (22°C, 70%RH 14L:10D photoperiod) where they were monitored for the emergence of parasitoids. Data for the three sites sampled in each year were pooled and the proportion of eggs collected which had been parasitised by each of the two primary parasitoid species was determined.

Behavioural Observations.

Thirty pairs of 1 x *E. nassau* and 1 x *N. insectifurax* were given access to an unparasitised batch of 10 *P. charybdis* eggs and their behaviour observed. All wasps were three-days-old, honey-fed and assumed to have mated. As these two species show no sexual dimorphism each wasp was allowed contact (in the absence of competition) with a batch of *P. charybdis* eggs 2 h before the observation to ensure only female wasps were selected. Females were identified as those which raised the abdomen to insert the ovipositor into a host egg, at which point the wasp was immediately removed before an egg could be laid.

The range of behaviours displayed by the two species was determined during exploratory observations and built into a configuration within *The Observer* software (Noldus Information Technology, Version 5). Each observation was conducted at ambient room temperature (20-27°C) under a dissecting microscope (Stemi SV6, Zeiss, Germany) at 8 – 12 x magnification for the duration of 30 min. Behaviours were recorded in real time using *The Observer* on a hand held PC. *Paropsis charybdis* eggs which were successfully parasitised were marked on an 'egg map' drawn at the beginning of each observation. Upon completion of each observation eggs which had not been parasitised were pricked with a pin to prevent *P. charybdis*

larvae hatching and eating the remaining eggs. Eggs were incubated (22°C, 75%RH, 14L:10D) until progeny emerged and could be identified.

RESULTS.

Field Parasitism.

Parasitism of *P. charybdis* eggs varied between sites but the combined total generally increased over the summer period from November to March each year (Fig. 1). Substantially more eggs were parasitised by *E. nassau*i. However, by late summer, i.e. February and March, *N. insectifurax* became the most abundant parasitoid for two of the three seasons (2002-2003 and 2004-2005). At one particular site (Rotomahana) almost 100% of *P. charybdis* eggs were parasitised by *N. insectifurax* in 2002-2003 (see Jones & Withers 2003). This corresponded with a release of 900 wasps in the area.

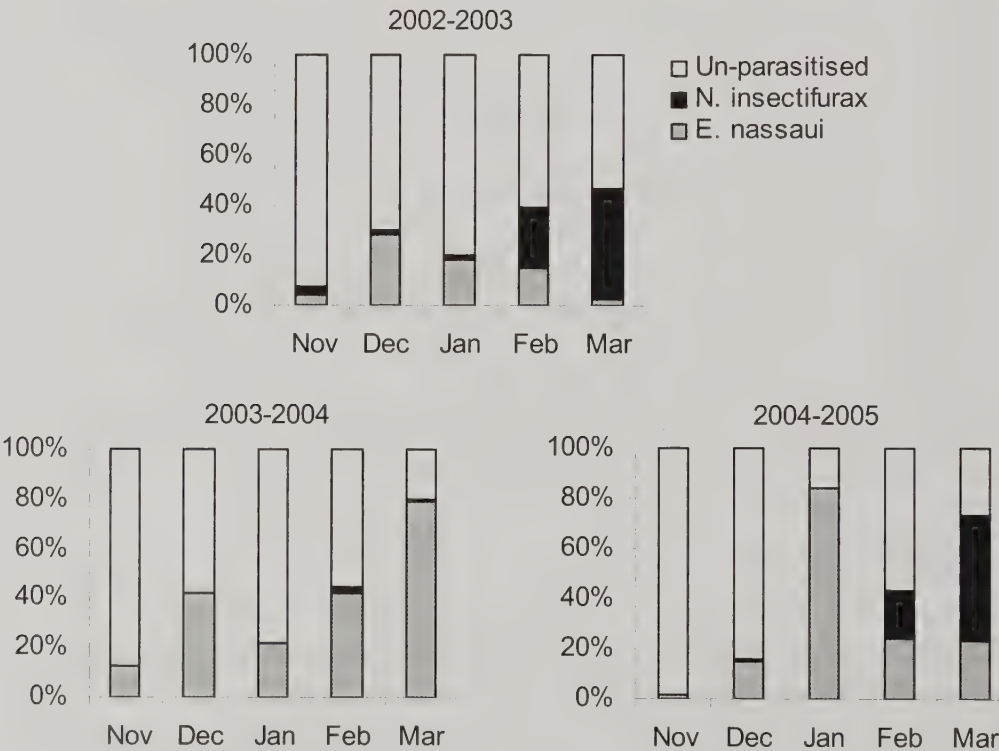


Fig.1. Proportion of field collected *Paropsis charybdis* eggs which were either un-parasitised or were parasitised by *Neopolycystus insectifurax* or *Enoggera nassau*i during the summer months from 2002 to 2005.

Behavioural Observations.

The mean proportion of time spent in contact with host eggs was 87% \pm 3.47% for *N. insectifurax* and 30% \pm 5.26% for *E. nassau*i. *Neopolycystus insectifurax* achieved 118 ovipositions in 29/30 observations and displayed oviposition behaviours during 55% of its time in contact with the host (Fig. 2). During an additional 31% of this time *N. insectifurax* displayed aggressive behaviours. These included biting and chasing *E. nassau*i or watching it while patrolling around the host

eggs. *Enoggera nassau* did not instigate competitive interactions, generally ignoring *N. insectifurax* unless approached by it and then responding by running away. *Enoggera nassau* devoted almost all its time (> 93%) to oviposition behaviours when in contact with host eggs (Fig. 2). However, the aggressive behaviour of *N. insectifurax* repeatedly disrupted *E. nassau* so it was difficult for the latter to remain in contact with the hosts and successfully complete an oviposition (Murray unpub. data). As a result only 57 eggs were successfully parasitised by *E. nassau* and these ovipositions occurred in just 16/30 observations.

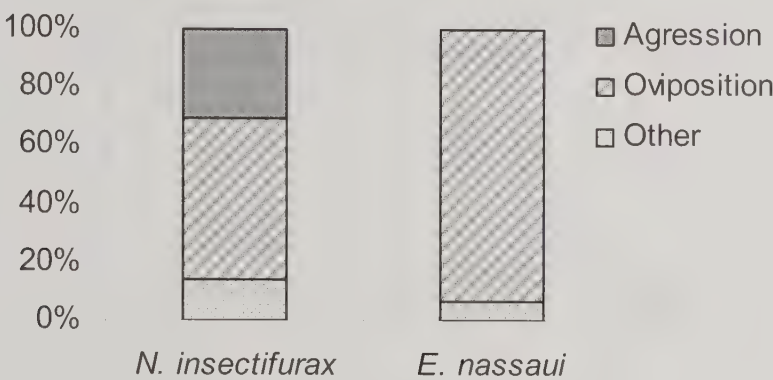


Fig. 2. Mean proportion of time in contact with *P. charybdis* eggs during which *E. nassau* and *N. insectifurax* displayed aggressive behaviours, oviposition behaviours or other behaviours in during 30 minute laboratory observations (n = 30 observations).

DISCUSSION.

Both *E. nassau* and *N. insectifurax* are capable of parasitising *P. charybdis* eggs in the laboratory at very high levels (90-100% Murray unpub. data). In the field however *E. nassau* has been found to parasitise substantially more eggs than *N. insectifurax* especially during the early part of the season. This probably results, in part, from the two species having slightly different environmental requirements (Tribe 2000). For example, the high level of parasitism achieved by *N. insectifurax* in one site in 2003 (Jones & Withers 2003) is unlikely to have resulted from the small release of the species in the area. Rather, a combination of factors such as particularly warm temperatures and abundant hosts at that time may have been responsible. Evidence presented here indicates that behavioural differences may also affect relative parasitism by the two species. Both will compete aggressively with conspecifics in the laboratory (Mansfield unpub. data) yet the slightly larger *N. insectifurax* is considerably more aggressive than *E. nassau* during interspecific competition. In our observations, parasitoids had access to only one patch of hosts. Therefore, when *E. nassau* was driven away from the hosts by *N. insectifurax* there was no opportunity to find and exploit a new patch. In the absence of competition in the laboratory, *E. nassau* completes oviposition faster than *N. insectifurax* (Mansfield unpub. data) and *N. insectifurax* has been observed to remain on a patch of hosts for a considerable time (over 24 h) after parasitising all the available eggs (Murray unpub. data). Under field conditions, while *N. insectifurax* defends one patch, *E. nassau* has the opportunity to search for and attack other patches. This may contribute to the higher parasitism rates by *E. nassau* in the field and at least partly

explain its broader geographic range in New Zealand compared with *N. insectifurax* (Murray *et al.* 2008).

CONCLUSIONS.

For *E. nassau* and *N. insectifurax*, aggressive defence of host resources in the presence of interspecific competition does not appear to improve biological control. We conclude that in the absence of *E. nassau*, *N. insectifurax* may make a poor biological control agent, providing insufficient control of *P. charybdis* unless parasitoid densities are very high. When both parasitoid species are present, however, the overall control of *P. charybdis* is probably greater than when exposed only to *E. nassau*.

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EXOTIC INTRODUCTIONS OF PRIMARY PARASITOIDS OF APHIDS IN NEW ZEALAND: THE GOOD AND THE BAD

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ABSTRACT.

This paper summarises the primary aphid parasitoid species (a mixture of intentionally introduced species, self-introduced species and probably indigenous species) recorded from introduced and indigenous aphids in New Zealand. It reviews the effectiveness of the intentionally and self-introduced primary parasitoids on aphid pests in New Zealand in terms of their ability to control pest aphid species and contrasts this with their potential impact on indigenous aphids.

The majority of aphids found in New Zealand have been inadvertently introduced (about 100 species), including many that are important plant pests in horticulture, agriculture, and forestry. In recent years a growing number of indigenous New Zealand aphid species (currently over 15) have been recognised that are of considerable scientific interest. Most of these indigenous species were discovered after the biocontrol introductions for pest species.

Of the primary parasitoid species purposefully introduced, several are considered to be effective biological control agents, e.g. *Aphidius rhopalosiphi* and *Aphidius ervi*. Additionally, other self-introduced species are also considered to be effective biological control agents, e.g. *Aphidius colemani*. There is increasing evidence that several intentionally and self-introduced primary parasitoid species are attacking indigenous aphid species in New Zealand and we are endeavouring to quantify their impact on these species. The lack of documented morphological characteristics for some species and the presence of undescribed indigenous genera/species in New Zealand make primary aphid parasitoid identification very difficult. The use of molecular techniques has in some part improved the reliability of identification.

INTRODUCTION.

Invasive aphids.

The majority of aphids found in New Zealand have been inadvertently introduced (about 100 species) (Teulon & Stufkens 2002). They have a significant economic impact on arable, vegetable, fruit and ornamental crops, and forest trees, and have unquantified impacts on natural systems. Aspects of their biology, such as their small size, parthenogenetic reproduction, high reproductive rate, short generation time, rapid dispersal and eruptive population dynamics pose difficult challenges for biosecurity and pest management in New Zealand (Teulon & Stufkens 2002).

Indigenous aphids.

Over 15 species of native aphids have also been recorded in New Zealand, with the majority of these being recognised in the last 10 years (Teulon et al. 2003; Teulon et al. unpublished data). They constitute a distinctive component of the world aphid fauna and New Zealand fauna, with species belonging to the Neophyllaphidinae, Taiwanaphidinae (primitive subtribes with Gondwanan distributions) and Aphidinae (mostly Aphidini). Recent molecular research places a group of native aphid species as central to the global evolution of the species-rich Aphidinae (von Dohlen & Teulon 2002) that includes many of the world pest species. New Zealand native species are not considered plant pests but those species in *Aphis/Paradoxaphis* and *Euschiaphis* genera are related to common pest species found in New Zealand. Aphid species native to New Zealand are generally monophagous (at least to plant genus) and are found on plant species bordering natural and productive systems.

Natural enemies.

The relatively narrow range of predators and parasitoids on aphids in NZ has been recognised for some time and as a result a number of predator (Coccinellidae, Chrysopidae, Hemerobiidae, Coniopterygidae, Chamaemyiidae and Syrphidae) and parasitoid (mostly Aphelinidae, Braconidae, see below) species have been introduced into New Zealand for aphid pest management (Thomas 1989).

We are interested in the impact of introduced parasitoids on indigenous aphids and how this might inform future introductions of biological control agents into New Zealand. The New Zealand aphid/parasitoid system provides a useful model for assessing the non-target impact of natural enemies due to; **(1)** the relatively large numbers (in terms of species and total numbers) of introduced compared with native aphids found in New Zealand, **(2)** the number of parasitoid introductions that were made before the size of the native aphid fauna was known, **(3)** the close taxonomic relatedness of some native and introduced aphids in New Zealand, and **(4)** the type of host plants of New Zealand aphids that are often found on the border between natural and productive systems.

In this paper we review the known primary parasitoid fauna in New Zealand, including both deliberate and unintentional introductions of exotic parasitoids, and attempt to place these introductions in the context of their potential non-target impact on native New Zealand aphids.

PRIMARY PARASITOIDS: INTENTIONAL INTRODUCTIONS.

There have been seven or eight successful introductions, and a small number of unsuccessful intentional introductions of aphid primary parasitoids to New Zealand. These introductions are summarised in Cameron et al. (1989). One further introduction has taken place since then. Valentine and Walker (1991) and Anonymous (2007) provide a list of species established in New Zealand:

Aphelinus mali (Haldeman) (Aphelinidae) for the woolly apple aphid (*Eriosoma lanigerum* (Hausmann)) on apple. Sourced from several states in the USA and released in New Zealand from 1921 to 1924 after screening for secondary parasitoids. It became widespread in New Zealand by 1925 and is considered to be a highly successful biocontrol agent except where interrupted by pesticide applications (Walker 1989).

Aphelinus subflavens (Westwood) (Aphelinidae) for the oak aphid (*Myzocallis annulatus* (Hartig)) on oak and chestnuts. Introduced to New Zealand from Europe via Australia in about 1939, but subsequently found to be present in New Zealand at that time. The oak aphid is no longer considered a significant problem, suggesting that *A. subflavens* was a successful biocontrol agent (Walker 1989).

Aphidius eadyi Stary, Gonzales & Hall (Braconidae) for the pea aphid (*Acyrtosiphon pisum* (Harris)) on lucerne. Originated from Morocco via California and released into New Zealand from 1977 to 1981 with subsequent distribution throughout New Zealand. It became established in all lucerne areas by 1987, with parasitism rates of 30-40% associated with declining pea aphid populations (Cameron & Walker 1989).

Aphidius ervi Haliday (Braconidae) for the bluegreen lucerne aphid (*Acyrtosiphon kondoi* Shinji) and pea aphid (*Acyrtosiphon pisum* (Harris)). Strains from various countries (via California), Australia and UK were released into New Zealand from 1977 to 1981. *A. eadyi* and particularly *A. ervi* have contributed, along with a range of other natural enemies and resistant cultivars, to the control of the bluegreen lucerne and pea aphid (Cameron *et al.* 1989). It should also be noted that a specimen considered to be *A. ervi* was identified in New Zealand in 1963 from *Aulacorthum solani* on *Histeropteris excelsa* (a fern) (M. Carver pers. comm. to J Berry).

Trioxys complanatus Quilis (Braconidae) for the spotted alfalfa aphid (*Therioaphis trifolii* (Monell)) on lucerne. Introduced into New Zealand from Australia from 1982 to 1985, established only in localised populations and has not been recovered since 1985. *T. trifolii* proved not to be a pest under New Zealand conditions (Walker & Cameron 1989).

Ephedrus plagiator (Nees ab Esenbeck) (Braconidae) was introduced from Japan via Australia for biocontrol of *A. kondoi* and *A. pisum* in 1977. Cameron *et al.* (1989) noted that field recoveries did not persist but this species is listed in the Checklist of New Zealand Hymenoptera as being in New Zealand (Anonymous 2007) (M. Carver, pers. comm.).

Aphidius rhopalosiphi De Stefani Perez (Braconidae) for the rose-grain aphid (*Metopolophium dirhodum* (Walker)) on cereals (esp. barley). Introduced from England and France and released in 1985 from 1987 with recoveries made from most cereal-growing areas in 1987 (Stufkens & Farrell 1989). This introduction was considered to be a success, with Grundy (1990) estimating that *A. rhopalosiphi* provided annual benefits of between NZ\$0.3 and \$5 million p.a. *A. rhopalosiphi* also parasitises another important cereal aphid pest found in New Zealand – *Rhopalosiphum padi* (L.).

Aphidius sonchi Marshall (Braconidae) for the sowthistle aphid (*Hyperomyzus lactucae* (L.)) on blackcurrant. Introduced from Australia in 1994 and established throughout New Zealand, except for the southern South Island). Stufkens & Farrell (1995) suggest it is was likely to be present in New Zealand at the time of release. Significant levels of parasitism were recorded on its secondary host (sowthistle), but minimal levels were recorded on its primary host (blackcurrant).

Additionally, *Aphidius smithi* Sharma & Subba Rao, *Ephedrus plagiator* (Nees ab Esenbeck), and *Praon barbatum* Mackauer were introduced for biocontrol of *A. kondoi* and *A. pisum* in 1977, although there was no confirmation of their establishment (Cameron *et al.* 1989; Valentine & Walker 1991 Anonymous 2007).

It appears that the parasitoid impact on non-target aphid species was not examined until the introduction of *A. rhopalosiphi* in 1985 when this parasitoid species was only tested on other introduced aphid species (Table 1). *Aphidius sonchi* was tested on four of the known six New Zealand native species before its introduction in 1994 (Stufkens & Farrell 1994). All introductions were made before the extent of the indigenous aphid fauna became apparent in the late 1990s.

Table 1. Deliberate introductions of primary parasitoids for biocontrol of aphids – confirmed establishment in New Zealand. Host record data are from Carver 1984, 2000 for Australia (in Waterhouse & Sands 2001).

Parasitoid species	Introd	Host aphid genera	Native aphids species (# tested)
<i>Aphelinus mali</i>	1921	<i>Eriosoma</i>	0 (0)
<i>Aphelinus subflavens</i>	1939	<i>Tuberculoides</i>	1 (0)
<i>Aphidius eadyi</i>	1977	<i>Acyrtosiphon</i>	6 (0 ^a)
<i>Aphidius ervi</i>	1977	<i>Acyrtosiphon</i> , <i>Macrosiphon</i> , <i>Metopolophium</i> , <i>Myzus</i>	6 (0)
<i>Trioxya complanatus</i>	1982	<i>Therioaphis</i>	6 (0)
<i>Aphidius rhopalosiphi</i>	1985	<i>Metopolophium</i> , <i>Rhopalosiphum</i>	6 (0 ^b)
<i>Aphidius sonchi</i>	1994	<i>Hyperomyzus</i>	6 (4 ^c)
			15+ ^d

Information on the number of New Zealand native aphids and number of species tested from: ^aCottier 1953, ^bUnpublished files (Crop & Food Research), ^cStufkens & Farrell 1994, ^dTeulon *et al.* submitted.

PRIMARY PARASITIDS: SELF-INTRODUCTIONS.

In addition to those primary parasitoids deliberately introduced into New Zealand for aphid biocontrol, a number of self-introduced species have established in New Zealand (Valentine & Walker 1991; Anonymous 2007) (Table 2).

Examples include:

Diaeretiella rapae (M'Intosh) (Braconidae) has been recorded in New Zealand since at least 1930 (Gourlay 1930) and is a parasitoid of *Brevicoryne brassica*. Its effectiveness as a biocontrol agent has been severely undermined by a number of hyperparasitoids (Cottier 1953; Thomas 1989).

Aphidius salicis Haliday (Braconidae) is a parasitoid of the carrot aphid (*Cavariella aegopodii* (Scopoli)), which has been recorded in New Zealand since at least 1962 (Carver & Stary 1974). While carrot aphid is a serious pest of carrots in New Zealand (Lowe 1971), there is little information on the impact of the parasitoid. However, in Australia the intentional introduction of *A. salicis* in 1962 and a change in the predominant carrot cultivar grown significantly reduced the pest status of the carrot aphid (Waterhouse & Sands 2001).

Aphidius colemani Viereck (Braconidae) is not recorded in Valentine & Walker (1991), but is found in Anonymous (2007). Unpublished records suggest it has been in New Zealand since at least 1982. It is an effective biological control agent for a range of aphid species in greenhouse crops and is commercially available in New Zealand (www.bioforce.net.nz, www.zonda.net.nz).

Praon necans Mackauer (Braconidae) is a central and eastern European species that attacks *Rhopalosiphum* and *Schizaphis* species. This species, or one very similar to it, has been found parasitising a New Zealand native *Euschizaphis* species (Mackauer pers. comm.), but has not been recorded from any introduced species in New Zealand.

There are also a number of unpublished records of the following exotic aphid parasitoid species in New Zealand: *Ephedrus persicae*, *Trioxys auctus*, *Trioxys cirsi*, *Trioxys compressicornis*, *Trioxys pallidus* and *Trioxys tenuicaudus*.

PRIMARY PARASITOIDS: INDIGENOUS APHIDS.

Field surveys.

Since about 1998 we have made observations on and collected parasitoids, including primary parasitoids, of native New Zealand aphids. Approximately 400 parasitoids have been collected from a range of native aphid species (Table 3). Most were collected in the South Island of New Zealand, where most native aphid populations have been found. We have concentrated our efforts on the New Zealand native *Aphis/Paradoxaphis/Casimira* group, which is closely related to many pest species.

DNA diagnostics.

Limited knowledge about the New Zealand aphid parasitoid fauna and the lack of available aphid parasitoid taxonomic expertise has hampered efforts to measure the non-target impacts of introduced biocontrol agents.

Table 2. Self-introductions of primary parasitoids – confirmed establishment in New Zealand. Host record data is from Carver 1984, 2000 for Australia (in Waterhouse & Sands 2001).

Parasitoid species	Host aphid genera	NZ reference
Braconidae		
<i>Aphidius colemani</i>	<i>Aphis</i> , <i>Myzus</i> , <i>Rhopalosiphum</i> , <i>Toxoptera</i>	Anonymous 2007
<i>Aphidius pelargonii</i>	<i>Acyrtosiphon</i> ^a	Valentine & Walker 1991
<i>Aphidius salicis</i>	<i>Cavariella</i>	Carver & Stary 1974
<i>Aphidius similis</i>	<i>Myzus</i> , <i>Rhopalosiphum</i>	Valentine & Walker 1991
<i>Diaeretiella rapae</i>	<i>Brevicoryne</i> , <i>Myzus</i> , <i>Rhopalosiphum</i>	Gourlay 1930, Cottier 1953
<i>Lysiphlebus testaceipes</i>	<i>Aphis</i> , <i>Rhopalosiphum</i> , <i>Toxoptera</i>	Anonymous 2007
<i>Praon</i> nr. <i>necans</i>	[<i>Euschizaphis</i>] ^b	Mackauer pers. comm.
Aphelinidae		
<i>Aphelinus abdominalis</i>	<i>Acyrtosiphon</i>	Valentine & Walker 1991
<i>Aphelinus asychis</i>	<i>Therioaphis</i>	Valentine & Walker 1991
<i>Aphelinus gossypii</i>	<i>Aphis</i> , <i>Macrosiphum</i> , <i>Myzus</i> , <i>Toxoptera</i>	Valentine & Walker 1991
<i>Aphelinus humulis</i>	<i>Aphis</i>	Valentine & Walker 1991

^aNew Zealand host record from Valentine & Walker (1991).

^bNew Zealand host record.

Our research group has been trialling a DNA sequence-based diagnostic technique for identifying aphid parasitoids to species level. A reference data set consisting of mitochondrial 16SrRNA sequences has been assembled. These data comprise available sequences from braconid and aphelinid taxa that appear in the New Zealand Arthropod Collection checklist of New Zealand hymenoptera (Anonymous 2007). New Zealand taxa not represented in public domain sequence databases are represented by sequences from congeners.

Wasp-specific primers (Jones *et al.* 2005) were used to detect the presence of a parasitic wasp in mummies collected from the field (see above). Amplified fragments were gel purified and sequenced. Following sequence editing, ClustalX (Thompson *et al.* 1997) was used to align mummy 16S sequences with those in the parasitoid reference set. PAUP* (Swofford 2000) was used to calculate a genetic distance matrix from which a neighbour-joining topology was generated.

Parasitoids on native aphids.

Results to date indicate that DNA sequence data is a useful tool for cataloguing the diversity of the New Zealand native aphid parasitoid fauna as a range of braconid and aphelinid species have been identified from our field surveys (Table 3). Some species have yet to be fully characterised and may be New Zealand native parasitoids new to science and other species need to be verified using morphological characters. The overall impact of any of these parasitoids on native aphid populations is unknown at this time

Introduced parasitoids have now been implicated attacking three New Zealand native aphid species: **(1)** *Aphidius* nr *ervi* on *Aphis cottieri* (Carver 2000), **(2)** Several *Aphidius* species on *Aphis healyi*, and **(3)** *Praon* nr. *necans* on a *Euschizaphis* species (M. Mackauer, pers. comm.). Given the taxonomic relatedness of several native aphid species to introduced aphid pest species and the polyphagous nature of several aphid parasitoids it is not unexpected that native aphids are attacked by introduced parasitoids. *A. ervi* is selectively polyphagous on the Macrosiphini but is also known to attack *Aphis* species (Marsh 1977). *Euschizaphis* is closely related to *Rhopalosiphum* and *Schizaphis* which are known hosts for *Praon necans* (M. Mackauer, pers. comm.).

Table 3. Primary aphid parasitoids attacking selected indigenous aphids in New Zealand.

Native aphid species	Primary parasitoid	Reference/comments
<i>Aphis coprosmae</i>	<i>Aphidius</i> sp. <i>Aphelinus</i> sp.	No aphids or parasitoids collected since 1997
<i>Aphis cottieri</i>	<i>Aphidius</i> nr. <i>ervi</i> <i>Aphidius</i> sp. ^a	Carver 2000
<i>Aphis healyi</i>	<i>Aphidius</i> sp. ^{a,b} <i>Aphidius</i> sp. ^a	
<i>Aphis nelsonensis</i>	None recorded	Aphid colonies not seen since 1965
<i>Aphis</i> sp. (on <i>Olearia</i>)	<i>Aphelinus</i> sp. ^a	
<i>Aphis</i> sp. (on <i>Hebe</i>)	No parasitoids recorded to date	Aphid colonies recently discovered 2008
<i>Paradoxaphis aristoteliae</i>	Not yet subjected to DNA analysis	
<i>Paradoxaphis plagianthi</i>	Not yet subjected to DNA analysis	
<i>Euschizaphis</i> spp. (<i>Dracophyllum</i>)	<i>Praon</i> nr. <i>necans</i>	Mackauer pers. comm.
<i>Euschizaphis</i> sp. (<i>Aciphylla</i>)	<i>Aphidius</i> sp. ^a <i>Lysiphlebus</i> sp. ^a	
<i>Neophyllaphis totarae</i> <i>Neophyllaphis</i> sp. (on <i>P. nivalis</i>)	New genus/species	Mackauer pers. comm.
<i>Sensoriaphis nothofagi</i>	None recorded	

^aParasitoid species confirmed by DNA sequence data.

^bPutative introduced species.

CONCLUSIONS.

A range of primary aphid parasitoid species (a mixture of intentionally introduced species, self-introduced species and probably indigenous species) are recorded from introduced and indigenous aphids in New Zealand. Both deliberately and unintentionally introduced parasitoids species make important contributions to the biological control of pest aphid species. Lack of knowledge of New Zealand native aphids and aphid parasitoids, including their taxonomy, is a severe impediment to understanding the impact of introduced parasitoids on native aphids and how this information might inform future introductions of biological control agents into New Zealand.

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SESSION 13

INDUCIBLE PLANT RESPONSES AND ITS IMPACT ON BIOLOGICAL CONTROL OF PLANT PESTS

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ABSTRACT.

Plants have evolved a broad arsenal of traits which provide them protection against herbivory. These traits are often inducible and based on their primary target are categorized as either direct or indirect resistance mechanisms. Direct resistance refers to plant traits that have a direct negative impact on herbivores, while indirect resistance target herbivore natural enemies. Plants have evolved a range of indirect resistance mechanisms including food supplements and shelter that support predator/parasitoid survival, as well as volatile signals that facilitate the locating and removal of herbivores. Recently we have seen an increased appreciation of the importance of such indirect interactions in shaping communities and in driving plant arthropod interactions. This session will focus on indirect interactions between plants and herbivores mediated by higher trophic level organisms. In addition to new insights in the functioning and dynamics of indirect resistance mechanisms, evidence for the impact of induced defenses on biological control efficacy will be presented and strategies to integrate induced plant responses in sustainable crop protection will be explored. Particular attention will be given to the fact that direct resistance mechanisms can have a substantial negative impact on higher trophic level organisms as well, while food, shelter and infochemicals may be exploited by herbivores. These ecological costs need to be addressed if we want to understand how plants optimize their resistance mechanisms. A number of examples in which induced defenses may be in conflict with biological pest control will be discussed.

INDUCTION OF SYSTEMIC ACQUIRED RESISTANCE IN ZEA MAYS ALSO ENHANCES THE PLANT'S ATTRACTIVENESS TO PARASITOIDS

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ABSTRACT.

Plants under attack by caterpillars emit volatile compounds that attract the herbivore's natural enemies. In maize, the caterpillar-induced production of volatiles involves the phytohormone jasmonic acid (JA). In contrast, pathogen attack usually up-regulates the salicylic acid (SA)-pathway and results in systemic acquired resistance (SAR) against plant diseases. Activation of the SA-pathway has often been found to repress JA-dependent direct defenses, but little is known about the effects of SAR induction on indirect defenses such as volatile emission and parasitoid attraction. We examined if induction of SAR in maize, by chemical elicitation with the SA-mimic benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), attenuates the emission of volatiles induced by *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) or exogenously applied JA. In addition, we determined how these treatments affected the attractiveness of the plants to the parasitoid *Microplitis rufiventris* Kokujev (Hymenoptera: Braconidae) in a six-arm-olfactometer. BTH treatment alone resulted in significant systemic resistance of maize seedlings against the pathogen *Setosphaeria turcica* (Luttrell) Leonard & Suggs (Pleosporales: Pleosporaceae), but had no detectable effect on volatile emissions. Induction of SAR significantly reduced the emission rates of two compounds (indole and (E)- β -caryophyllene) in JA-treated plants, whereas no such negative cross-talk was found in caterpillar-damaged plants. Surprisingly, however, BTH treatment prior to caterpillar-feeding made the plants far more attractive to the parasitoid than plants that were only damaged by the herbivore. Control experiments showed that this response was due to plant-mediated effects rather than attractiveness of BTH itself. We conclude that in the system studied, plant protection by SAR activation is compatible with and can even enhance indirect defense against herbivores.

PROSPECTS OF EXPLOITING HERBIVORE-INDUCED PLANT VOLATILES TO ENHANCE BIOLOGICAL CONTROL IN MAIZE

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ABSTRACT.

When plants are attacked by insect herbivores they respond by emitting specific volatile compounds, which have been shown to be highly attractive to natural enemies of these herbivores, such as predators, parasitic wasps and entomopathogenic nematodes. In maize, the volatiles emitted upon attack by leaf-chewing or root feeding arthropod herbivores have been particularly well studied in the laboratory and several key compounds mediating the described interactions have been identified so far. Moreover, several genes and biochemical pathways responsible for the production of the emitted volatiles have been elucidated. The advances in understanding the volatile emission and its ecological signaling open novel ways to modify volatile blends in order to enhance their attractiveness to natural enemies. Here we briefly describe different approaches that can be applied to exploit herbivore-induced volatile blends to enhance biological control of two important herbivores on maize. By using examples from our own work and from the work of others, we argue that most promising results might be obtained by manipulating the emission of specific volatile compounds, while simultaneously enhancing the responsiveness of natural enemies to these target compounds. Finally, as single herbivore-induced volatile compounds differ in their information value for the numerous herbivores and natural enemies usually found in crop fields we highlight the importance of taking a holistic ecological approach while evaluating risks and benefits of modified volatile blends.

INTRODUCTION.

Plant volatiles emitted upon infestation with some herbivores are important foraging cues for natural enemies of these herbivores. Numerous recent studies have provided convincing evidence that these volatiles indeed function as an indirect defense by which the plants purposefully recruit parasitoids and predators (for recent reviews: D'Alessandro & Turlings 2006; Heil 2008; Turlings & Wäckers 2004). Maize, *Zea mays* L. (Poaceae), has been a model plant system since the very beginning of the studies on these chemically-mediated tritrophic interactions (e.g. Turlings *et al.* 1990) and the volatile blends emitted from the aboveground shoot and the belowground roots are well characterized (D'Alessandro & Turlings 2005; Rasman & Turlings 2008). Some of the most intriguing ecological aspects of herbivore-induced maize volatiles are illustrated in Fig. 1. However, volatile compounds of herbivore infested maize plants are not emitted in consistent blends but quality and quantity vary, depending on time and type of infestation, plant developmental stage

and a series of other biotic and abiotic factors (Gouinguéné & Turlings 2002; Köllner *et al.* 2004; Rostás *et al.* 2006; Turlings *et al.* 1998). This surprisingly high complexity and variability of herbivore-induced maize volatiles is probably part of the reason why the role of individual compounds for the attraction of different natural enemies is still poorly understood. Yet, for some parasitoid species of *Spodoptera* (Lepidoptera: Noctuidae) caterpillars we now know that several of the compounds emitted in high quantities upon infestation by these herbivores may mask the attractiveness of other highly attractive compounds emitted in only minor quantities and others may even be repellent (D'Alessandro & Turlings 2005; D'Alessandro *et al.* 2006).

A very specific and highly attractive compound was recently discovered in studies on maize roots damaged by the ferocious pest *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). Larvae of this beetle induce maize roots to emit the sesquiterpene (*E*)- β -caryophyllene (Rasmann *et al.* 2005). In laboratory studies it was not only shown that synthetic (*E*)- β -caryophyllene is highly attractive to the entomopathogenic nematodes but also that it is an ideal compound to diffuse through the complex belowground soil compartment (Hiltpold & Turlings 2008). The important role of this compound in the indirect defenses of maize was further confirmed in the field by comparing the attractiveness of maize varieties that release the signals with varieties that have lost this ability. Most striking were the results from subsequent field experiments in which a 5-fold higher nematode infection rate was found for larvae near a variety that emitted (*E*)- β -caryophyllene than near a variety that does not release the compound (Rasmann *et al.* 2005).

Besides the detailed knowledge on the ecological importance of these volatile-mediated interactions, the understanding of the molecular and physiological aspects of the induction and biosynthesis of herbivore-induced maize volatiles has also progressed over the last few years. For example, it was found that the odor emission from the aboveground shoot of maize seedlings is not merely the result of mechanical damage to the leaf tissue, but is elicited by compounds in the oral secretions of the caterpillars (Alborn *et al.* 1997; Turlings *et al.* 1993a). Several elicitors have been isolated so far (Schmelz *et al.* 2006; Tumlinson & Lait 2005; Voelckel & Baldwin 2004) and they may be partially responsible for the fact that some plants have the ability to detect which herbivore is feeding on them and to respond accordingly by emitting volatile compounds which are highly specific and highly attractive to predators and parasitoids of the attacking herbivore (De Moraes *et al.* 1998; Felton & Tumlinson 2008). Moreover, several plant hormones, biochemical pathways and genes involved in the volatile emission in maize have been identified (Frey *et al.* 2000; Schmelz *et al.* 2003; Schnee *et al.* 2002) and the elucidation of their relevance for the functioning of tritrophic interactions has now been started (Köllner *et al.* 2008; Schnee *et al.* 2006).

The enormous advances in research on molecular mechanisms and ecological signaling of herbivore-induced maize volatiles open exciting novel prospects of manipulating the release of these compounds in order to make maize plants more attractive to natural enemies. Here we summarize various approaches that can be applied in the field to enhance biological control and we provide evidence from laboratory and field experiments that such approaches are indeed promising to develop novel sustainable crop protection strategies.

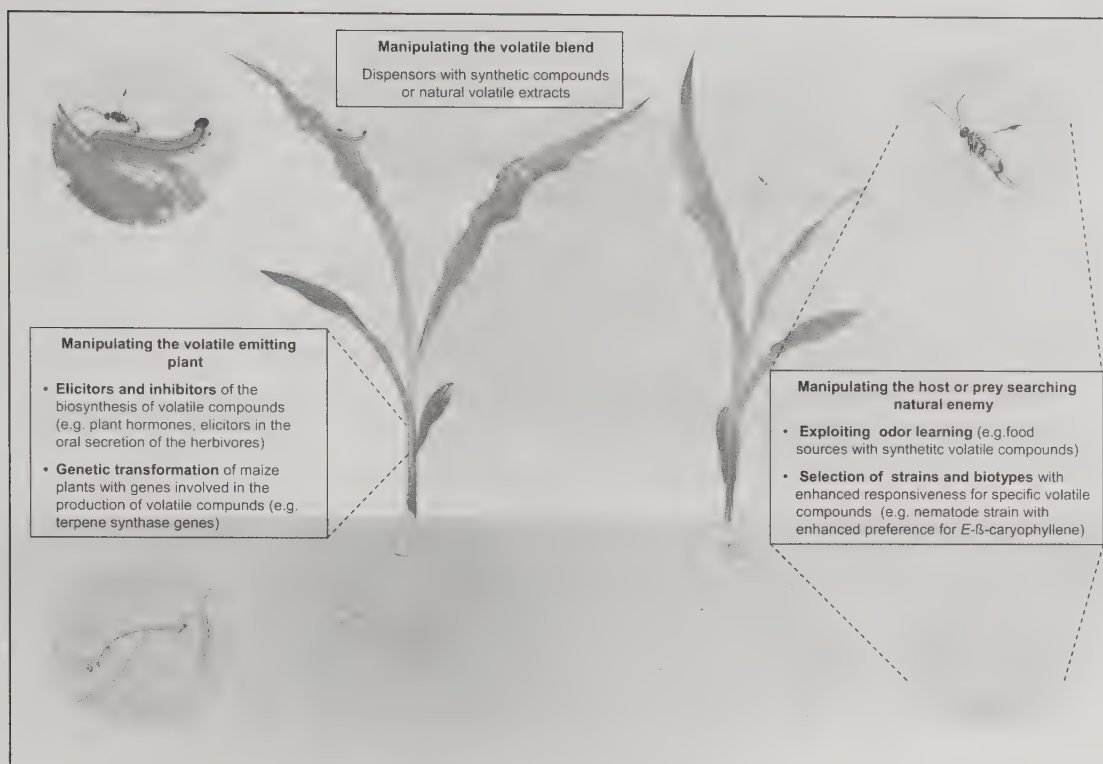


Fig.1. Young maize plants, when damaged by caterpillars immediately release several typical 'green leaf volatiles' from the damaged sites (indicated in green). In addition, elicitors in the caterpillar's oral secretion cause the induction of a systemic release of volatiles that mainly comprise terpenoids, but also some phenolics, such as indole (indicated in blue). This blend of volatiles is highly attractive to various parasitic wasps that lay their eggs in the caterpillars. Belowground, beetle larvae may cause the emission of similar signals by damaged roots (indicated in red). Maize roots release one dominating compound, (*E*)- β -caryophyllene, in response to root feeding. This sesquiterpene was found to be attractive to entomopathogenic nematodes and increase the effectiveness of these nematodes in finding and killing herbivore larvae. In addition, the herbivore-induced volatile blend may repel other herbivores and can induce or prime defense responses in neighboring plants. This figure has been taken from Turlings & Ton (2006) and different approaches that are suitable to exploit herbivore-induced plant volatiles to enhance biological have been added in the boxes.

MANIPULATING THE VOLATILE BLEND.

Probably the most straightforward approach to exploit herbivore-induced plant volatiles as novel crop protection agents is to apply dispensers emitting synthetic volatiles that are highly attractive to the natural enemies of the herbivorous pest insects in the field. This not only results in the recruitment of more beneficial insects into the field, but eventually also in a reduction of the damage caused by the insect

pest and in enhanced yields of the crop. Evidence that synthetic herbivore-induced plant volatiles indeed attract natural enemies in nature comes from a pioneering ecological field study by Kessler & Baldwin (2001) and has since then also been confirmed in more agricultural settings. For example, hop yards baited with methyl salicylate resulted in a significant increase in the numbers of beneficial predatory insects and in a dramatic reduction in spider mite numbers, a major arthropod pest of hops (James & Price 2004). In further field tests with synthetic herbivore-induced plant volatiles, 11 insect species or families showed significant attraction to 13 of the 15 tested volatile compounds (James 2005). In maize fields, Khan and colleagues (1997) nicely demonstrated that intercropping the fields with the odorous grass *Melinis minutiflora* P. Beauv. (Poaceae), which emits compounds that are typically released by maize in response to caterpillar damage, such as (*E*)- β -caryophyllene and (*E*)-4,8-dimethyl-1,3,7-nonatriene, resulted in largely reduced damage by a lepidopteran stemborer, partly because the pest was repelled by the odor of the grass, but also because one of its parasitoids was attracted to the mixed fields, leading to high parasitism rates (Khan *et al.* 1997). Several of these so called "push-pull" strategies have been developed over the past few years and the technology is now being widely used by farmers in Africa for the control of cereal stemborers, such as *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) (Cook *et al.* 2007; Khan *et al.* 2008).

Success or failure of manipulating odor blends with synthetic or natural volatile compounds certainly depends on whether the right compounds are added and whether natural enemies responding to these compounds are present in the field or not. Unfortunately, in most cases neither composition and dynamics of natural enemy populations of a specific pest herbivore, nor the most attractive compounds for these beneficials are known (but see for example De Boer & Dicke 2004). Therefore, one way to improve the efficacy of pest control by augmentative release of volatile compounds consists by first identifying which compounds have the greatest potential to attract the most dominant natural enemies of a certain pest insect in a given crop field. Identifying such key compounds is not an easy task, as herbivore-induced volatile blends are usually emitted in very complex and variable blends (D'Alessandro & Turlings 2006). To determine which compounds of the volatile blend emitted by maize seedlings infested with various species of *Spodoptera* caterpillars (Lepidoptera: Noctuidae) are the most attractive ones for *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae), a major parasitoid of these pest insects in many parts of the Americas, we developed several approaches in our laboratory (Held *et al.* 2006). For instance, we successfully isolated highly attractive compounds for *C. marginiventris* by passing a blend of herbivore-induced volatiles over filter tubes containing different adsorbents (D'Alessandro & Turlings 2005). One fraction that was retained on a silica filter and contained less than 30 % of all emitted compounds (*silica extract*) was of particular interest. In detailed olfactometer assays it was confirmed that the compounds of this silica extract were indeed essential and highly attractive for *C. marginiventris*, making them potential candidates to be released in the field in order to enhance conservation biological control against *Spodoptera* caterpillars (D'Alessandro & Turlings 2005). Here we show that adding a small amount of the attractive silica extract to freshly infested maize seedlings was sufficient to increase the attraction of *C. marginiventris* (Fig. 2A). This higher attraction was not due to higher amounts of major volatiles in the supplemented blends, but rather to minor qualitative differences in the supplemented blend. In fact,

volatile analyses indicated that the volatile blends added on filter paper evaporated within minutes, resulting in quantitatively identical blends emitted from infested plants with or without supplemented silica extract (volatile analysis not shown in this paper). The enhanced attractiveness of the supplemented odor source was most evident when parasitoids had an oviposition experience on larvae feeding on maize plants prior to the experiment. In fact, *C. marginiventris* females, like many other female parasitoids, are able to learn and associate plant volatiles with the presence of suitable hosts during oviposition experiences and to change their odor preference accordingly (D'Alessandro *et al.* 2006; Turlings *et al.* 1993b; Vet *et al.* 1995). The enhanced attraction to supplemented odor sources was lost when plants were infested for 26 to 30 h (Fig. 2B). One explanation for that would be that the latter plants emitted a series of non-attractive compounds or even repellent compounds that might have masked the attractiveness of the supplemented silica extract. In fact, gas-chromatographic analysis of the volatile blends emitted by freshly damaged and old damaged maize seedlings differ greatly in terms of quality and quantity (data not shown in this paper, but see (Hoballah & Turlings 2005). An additional indication that this was probably the case came from the observation that the responsiveness of the parasitoids to the odor of plants infested for 26 to 30 hr was lower than to the odor of freshly infested plants (Fig. 2). The attractive compounds in the silica fraction remain to be identified, after which it should be possible to test if they can enhance the recruitment of *C. marginiventris* in the field.

MANIPULATING THE VOLATILE-EMITTING PLANT.

Another promising approach to exploit herbivore-induced maize volatiles for enhanced biological control of *Spodoptera* caterpillars consists of manipulating the volatile blend emitted by plant. This can be achieved by treating plants with plant hormones and with inhibitors or elicitors that affect the biosynthesis of plant volatiles. This approach seems especially useful when key attractive compounds are unknown for a given natural enemy. For instance, Thaler (1999) treated tomato plants with jasmonic acid to induce typical emissions of herbivore-induced volatiles and observed that parasitism of lepidopteran larvae was significantly higher on treated plants than on untreated plants. In a laboratory study with maize, application of the salicylic acid-mimic benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) not only resulted in significant systemic resistance of maize seedlings against the fungal pathogen *Setosphaeria turcica* (Luttrell) Leonard et Suggs (Dothideales: Dothideaceae), but upon caterpillar damage the BTH treated plants were also far more attractive to the parasitoid *Microplitis rufiventris* Kok. (Hymenoptera: Braconidae) than plants that were only damaged by the herbivore (Rostás & Turlings 2008). Control experiments showed that this response was due to plant-mediated effects rather than to the attractiveness of BTH itself. Similar results were found in a recent field study in Mexico by comparing parasitism rates of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) feeding on plants that were treated with BTH to parasitism rates of larvae on control plants (von Mérey & D'Alessandro, unpub. data).

In cases where highly attractive volatiles are known and where genes involved in the biosynthesis of these compounds have been identified, breeders should now be able to manipulate the production of attractants through genetic transformation. The feasibility of this approach was first demonstrated in *Arabidopsis thaliana* (L.)

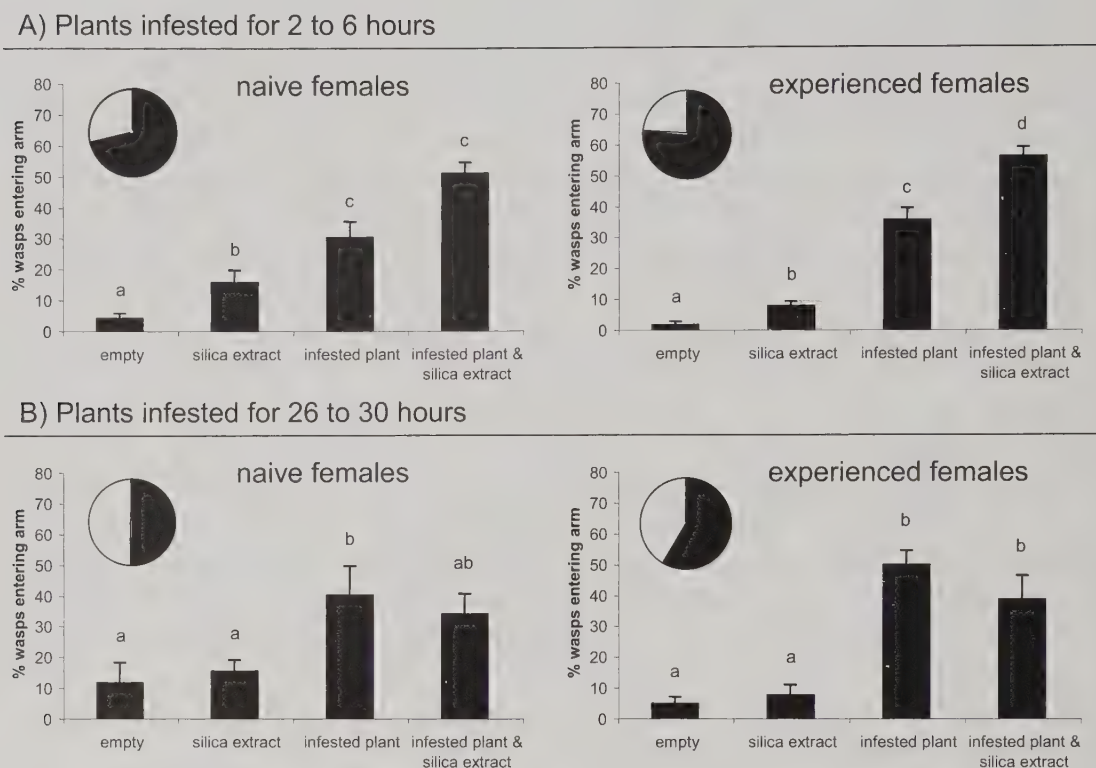


Fig. 2. Responses of *C. marginiventris* females to different odor sources in a four-arm olfactometer. Wasps had the choice between an empty arm with solvent as a control (*empty*), an arm with small amounts (100 μ l) of a minor fraction of herbivore-induced maize volatiles (*silica extract*), an arm with a *Spodoptera*-infested maize seedling (*infested plant*), and an arm with a *Spodoptera*-infested maize seedling supplement with the silica extract (*infested plant & silica extract*). **Experienced wasp** had oviposition experiences on *Spodoptera*-caterpillars while simultaneously being exposed to odors emitted by a *Spodoptera*-infested maize seedling 1 h prior to the olfactometer experiments. **Naive wasps** had no oviposition experiences and were not exposed to host or plant volatiles. **A)** *Spodoptera* caterpillars were kept feeding on the plants for 2 to 6 h. **B)** *Spodoptera* caterpillars were kept feeding on the plants for 26 to 30 h. Each experiment was replicated 8 times at different experimental days with 24 wasps per replicate. The **responsiveness** (proportion of wasps choosing an arm) is indicated by the **pie charts** with the white part representing wasps that did not enter any olfactometer arm. Further details on experimental and statistical procedures are given in D'Alessandro & Turlings (2005) and D'Alessandro *et al.* (2006).

Heynh. (Brassicaceae) plants (Kappers *et al.* 2005). Kappers and co-workers introduced a linalool/nerolidol synthase gene (FaNES1) from strawberry into *A. thaliana*, causing the transformed plants to constitutively release (3S)-(E)-nerolidol (and in some transformants (3E)-4,8-dimethyl-1,3,7-nonatriene), which rendered them attractive to predatory mites. In another example, *Arabidopsis* was transformed

with a maize gene (TPS10) that is responsible for the emissions of a sesquiterpene blend that is typically released in response to caterpillar feeding (Schnee *et al.* 2006). The transformed plant was attractive to *C. marginiventris*, but only after the wasps had learned to associate the sesquiterpene blend with the presence of hosts. Another gene that has been recently identified in maize is the terpene synthase gene TPS23, which is responsible for the production of (*E*)- β -caryophyllene, a key attractant for some entomophagous nematodes (Köllner *et al.* 2008; Rasmann *et al.* 2005). This gene is active in teosinte species and European maize lines, but decreased transcription in most North American maize lines resulted in the loss of (*E*)- β -caryophyllene production (Köllner *et al.* 2008). The amplification of the transcription of this gene or the introduction of an active gene from another plant species into the American maize lines, are interesting approaches, which might help to restore the attractiveness of the American maize lines to entomophagous nematodes. Fortunately, there still exist considerably high genetic variability in the emission of herbivore-induced maize volatiles (Degen *et al.* 2004) and therefore it should also be possible to incorporate highly attractive volatile compounds into new varieties using classical breeding programs.

MANIPULATING THE HOST OR PREY SEARCHING NATURAL ENEMY.

So far, relatively little effort has been invested in manipulating the responsiveness of natural enemies towards herbivore-induced volatiles. However, from the data and examples we presented in this paper it already becomes clear that natural enemies are not attracted in a fixed manner towards plant volatiles, but rather change their responsiveness depending on their physiological state, as for instance through associative odor learning (Fig. 2). This offers an additional way to enhance the responsiveness of natural enemies to certain volatile compounds. Several studies have reported amazing learning skills of certain parasitoids in host location (reviewed by Steidle & van Loon 2003). Moreover, learning of odor cues is not only important during oviposition experiences but also during food intake (Takasu & Lewis 1996; Wäckers *et al.* 2002). In fact, it has long been recognized that the efficacy of natural enemies as biological control agents against insect pests can be increased by supplying them with food sources (reviewed by Cortesero *et al.* 2000; Wade *et al.* 2008). We speculate that combining supplemented food sources with synthetic herbivore-induced plant volatiles would further enhance the foraging efficacy of certain natural enemies, such as parasitoids. This approach could be particularly successful for biological pest control in young maize fields that usually lack food sources for parasitoids.

In cases where augmentative biological control is an option for pest control the natural enemies themselves provide an additional target for manipulation. Integrating odor detection and attraction into rearing programs of a specific natural enemy could result in biological control agents with significantly improved host finding capacity. For instance, already after one generation of bi-directional selection of the generalist parasitoid *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) on a volatile extract from herbivore-induced *Brassica* plants it was possible to select two strains that significantly differentiated in both, flight orientation and landing success towards the tested odor source and their divergence continued upon further selection (Wang *et al.* 2003). In another study, it was shown that this bi-directional selection procedure

had neither a significant effect on the development time of the parasitoid larvae, nor on the body size and the number of female wasps per brood (Wang *et al.* 2004). By contrast, female wasps from the high olfactory-response strain were able to parasitize more host larvae in a wider area of habitats than the low olfactory-response strain. In the belowground interactions of maize roots damaged by *D. v. virgifera*, the identification of (*E*)- β -caryophyllene as a key compound for the attraction of entomopathogenic nematodes has opened the way to improve the responsiveness of nematodes to this sesquiterpene. Indeed, we recently selected a strain that migrates faster towards (*E*)- β -caryophyllene and thus might be more efficient in eliminating root-feeding beetle larvae (Hiltpold *et al.* unpub. data).

CONCLUSIONS.

Laboratory research on interactions between plants, herbivores and their natural enemies has made enormous progress over the last few years, not least because many groups working in the field of chemical ecology have adopted modern methods of biochemistry and molecular biology. By contrast, field studies that convert these findings and theories into practice remain scarce. Here we described different approaches that would allow for integrating herbivore-induced plant volatiles into modern pest control in maize. Molecular techniques to modify the volatile blend, such as transforming maize plants with certain genes that result in the release of specific attractive compounds, might also find their way into biological control. However, it should be stressed that herbivore-induced plant volatiles play a role in multiple interactions and might affect non-target herbivores and natural enemies as well as their neighboring plants (Turlings & Ton 2006). Therefore, the way forward seems to be to further enhance the specificity and efficacy of herbivore-induced plant volatiles. This could be achieved by combining several of the approaches described above, as for instance, manipulating the emission of single targeted volatile compounds, while simultaneously enhancing the responsiveness of natural enemies to this new compound. To test this hypothesis, the nematode strains which we selected to be particularly responsive to (*E*)- β -caryophyllene could now be released to increase biological control of *Diabrotica* larvae feeding on maize seedlings that emit (*E*)- β -caryophyllene in high quantities. Rigorous ecological field studies will be needed to evaluate whether this approach will indeed provide a sustainable and valuable alternative to conventional pest control.

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FOOD FOR PROTECTION: HOW PLANTS OPTIMIZE DEFENSIVE FOOD SUPPLEMENTS

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ABSTRACT.

Extrafloral nectaries are nectar glands found on plants structures other than flowers. They have evolved numerous times independently and are found in a broad range of plant families. They are commonly seen as an indirect defense mechanism, allowing plants to recruit ants or other predacious arthropods, which in turn protect the plant from herbivores. While such a defensive mutualism is an appealing interpretation, the issue whether the primary function of extrafloral nectar is defensive is often disputed. Here we use cotton as a model to test whether patterns of extrafloral nectar production optimize the costs and defensive benefits to the nectar secreting plant. It is shown that the temporal and spatial pattern in which cotton allocates extrafloral nectar production fits the predictions of the optimal defense theory. Moreover, plants actively adjust their food provision in response to herbivore damage and nectar removal. Furthermore, it is shown that the extrafloral nectar trait has been lost in cotton species found in ecosystems where the defensive function has become obsolete. Overall, these findings lend strong support to the defensive function of cotton extrafloral nectar.

INTRODUCTION.

Plants possess a broad range of traits which provide protection against herbivory (Karban & Baldwin 1997). In addition to those plant characteristics which act *directly* upon herbivores, plants can also be protected *indirectly*, as plants are believed to have evolved various adaptations which allow them to actively recruit herbivore natural enemies. These adaptations include the production of volatiles that indicate the presence of (feeding) herbivores, as well as the provision of shelter (domatia) and food rewards to accommodate the enemies of herbivores (Turlings & Wäckers 2004). Here we focus on extrafloral nectaries, which have been described in more than 1000 species from 93 plant families. These include numerous dicotyledonous species, ferns, and such diverse monocotyledonous taxa as lilies, orchids, sedges, and grasses (Koptur 1992). By producing extrafloral nectar, plants can recruit a range of predators, most prominently ants. In return, plants may obtain protection against herbivores, thus qualifying these extrafloral nectar mediated interactions as a defensive mutualism. In the case of ant recruitment, extrafloral nectar mediated interactions can extend to a range of other benefits because ants can provide nutrition or remove pathogens, and competing plants (Beattie 1985).

While it might be appealing to interpret traits that act upon herbivore natural enemies as 'indirect defense', other non-defensive interpretations have to be considered as well. Extrafloral nectaries have long been interpreted as 'sugar valves'

through which plants excrete surplus sugars (Bentley 1977; Bory & Clair Maczulajtys 1986; Rhyne 1965).

There are a number of ways to test whether plant traits actually do represent direct or indirect defense mechanisms. Firstly, we can test whether putative defensive traits actually translate into reduced herbivory and increased plant reproductive fitness. A second approach is to test whether patterns of trait expression optimize the defensive function (McKey 1974; Zangerl & Rutledge 1996).

To study extrafloral nectar production in terms of defensive function we have worked with *Gossypium* spp. (Malvaceae) as model plants. This choice is based on the following considerations: (1) *Gossypium* species possess a range of extrafloral nectaries both on their leaves, as well as on their bracts; (2) Production of extrafloral nectar in cotton is determined by 2 recessive genes, *ne1* and *ne2*, that are independent of the inheritance of floral nectar (Rhyne 1965); and (3) *Gossypium* species present a range of (inducible) direct and indirect defenses, allowing us to study complex interactions between various putative defenses. This paper gives an overview of the costs and defensive benefits of extrafloral nectar production and explores whether the temporal and spatial pattern in which cotton species allocate extrafloral nectar optimizes the cost-benefit function.

DEFENSIVE BENEFITS.

The fact that plants obtain a defensive benefit from predatory insects visiting extrafloral nectaries and/or food bodies was first proposed by Delpino (1874) and Belt (1874). The actual protective function of extrafloral nectaries was demonstrated by von Wettstein (1889). Excluding ants from bracteal nectaries on the flowering heads of two Compositae species, he was able to show that ant-tended plants suffered less damage to seeds by beetles and hemipteran bugs. For almost a century following these seminal publications, the concept of food as an indirect defense mechanism was widely discarded, before Janzen (1966) and others in the 1960's revived the idea. Through extensive experimental work, they were able to substantiate the fact that ants recruited to extrafloral nectaries and food bodies can benefit plant fitness. Over the last 50 years, the defensive fitness benefits of extrafloral nectar have been established in a broad range of systems (Heil *et al.* 2001; Oliveira 1997).

In wild cotton, it has been recently demonstrated that ants visiting foliar nectaries effectively protect plants from caterpillar damage (Rudgers 2003), ultimately enhancing seed production (Rudgers 2002). Ant recruitment by bracteal nectaries reduces fruit damage and fruit abortion rates in wild cotton, suggesting that bracteal as well as foliar extrafloral nectaries function in defense (den Held and Wäckers, unpublished). Moreover, ants recruited to bracteal or calyx nectaries may protect flowers from nectar robbers (Inouye 1983), or may promote outcrossing by reducing the time pollinators spend visiting flowers of a particular plant (Altshuler 1999). Ants may also have negative impacts on plant fitness when they rob floral rewards and/or castrate flowers, generally without contributing to pollination (Beattie 1985). Extrafloral nectar may serve to prevent these problems by distracting ants away from delicate flowers.

DEFENSIVE COSTS.

Producing nectar represents a direct cost in terms of invested metabolites. The often copious nectar volume secreted by extrafloral nectaries frequently exceeds floral nectar production both in terms of nectar production rate and duration of production. Individual leaves of *Ricinus communis* L. (Euphorbiaceae) excrete 3.6 mg of sugar per day, representing 1% of the leaf's daily assimilate production (Wäckers *et al.* 2001). Bractal nectaries of *Gossypium hirsutum* L. (Malvaceae) excrete up to 12 mg of highly concentrated nectar per fruit per day (Wäckers & Bonifay 2004). One hectare of cotton represents a daily production of 3.8 L extrafloral nectar. Nectar production is even higher in Bull's horn acacias that have been reported to produce 1 ml per nectary per day (Janzen 1966). Nevertheless, the physiological costs of extrafloral nectar production are thought to be limited, and a direct fitness cost of extrafloral nectar production remains to be demonstrated.

Probably more substantial of a cost factor are ecological costs in instances when extrafloral nectar is exploited by herbivores. In a number of cases it has been shown that nectar production increases a plant's chance to be selected as an oviposition site (Wäckers *et al.* 2007). The fitness costs of oviposition can be particularly high in species where females cluster their eggs and/or show oviposition aggregations. When herbivores oviposit in direct proximity to nectar sites, this may not only result in enhanced herbivore pressure on the nectar-producing plant itself but also on plants in its direct vicinity. These two scenarios likely have opposing fitness consequences for the nectar-producing plant. Whereas the former scenario may represent substantial fitness costs, the latter scenario may actually generate competitive benefits to the flowering plant when recruited herbivores effectively weaken the competitive strength of its neighbors (Wäckers *et al.* 2007).

Whereas defensive costs are often difficult to quantify, the fact that plants may lose extrafloral nectaries in ecosystems where the defensive function has become obsolete (see below) underlines that this trait represents substantial costs.

DO PATTERNS OF NECTAR PRODUCTION OPTIMIZE THE DEFENSIVE FUNCTION?

Assuming that extrafloral nectar has a defensive function, we can use the 'Optimal Defense Theory' (ODT) to test whether the pattern in which extrafloral nectar is produced and allocated optimizes the trade-off between costs and defensive benefits (McKey 1974; Zangerl & Rutledge 1996). Plants are predicted to allocate extrafloral nectar in ways that reflect (1) the ecology of the intended mutualists as well as possible nectar feeding herbivores, (2) the value of the plant tissue, and (3) the likelihood that a tissue will be attacked (Zangerl & Rutledge 1996).

Extrafloral Nectar Production and the Ecology of Nectar Consumers.

Patterns of extrafloral nectar production have been shown to match presence of nectar consuming ants. (Pascal and Belin-Depoux 1991) showed that daily nectar production patterns correlate with the diurnal activity pattern of tending ants. We see the most extreme adaptation in plants that have lost the extrafloral nectar trait in ecosystems where ants are less abundant or absent (Sugiura *et al.* 2006; Wäckers

and Bonifay 2004). An example is the Hawaiian cotton species *Gossypium tomentosum* Nutt. ex Seem. (Malvaceae) which is one of only two wild cotton species lacking extrafloral nectaries. The loss of this trait can be explained by the fact that the Hawaiian archipelago until recently lacked ants. The second species lacking extrafloral nectar, *Gossypium gossypoides* (Ulbr.) Standl. (Malvaceae), is found in Mexican highlands. At the given altitudes ants are less abundant, in comparison to the coastal ecosystems typically associated with *Gossypium* spp. (Wäckers & Bonifay 2004).

The positioning and morphology of extrafloral nectaries may also represent adaptations to nectar consumers. Extrafloral nectaries are often strategically positioned to enhance chances of being encountered by ant scouts (Bentley 1977). By far the majority of extrafloral nectaries are easily accessible and fully exposed, typically occurring on the surface of leaves, stipules, stems, or fruits (Koptur 1992). This allows exploitation by ants, as well as most other predators and parasitoids, as they often lack elongated mouthparts.

Like floral nectar, extrafloral nectar is typically dominated by sucrose and its hexose components glucose and fructose (Wäckers 2005). Although herbivory can induce nectar secretion (see below), it does not necessarily affect the sugar concentration or sugar composition of the secreted nectar (Wäckers *et al.* 2001). In contrast to floral nectar, extrafloral nectar often has higher overall sugar concentration, as well as increased fructose and glucose levels. The high sugar concentration may support a defensive function (Wäckers *et al.* 2001). High sugar concentrations reduce intake by visiting ants, prolong ant visits (Josens *et al.* 1998), and help prevent nectar use by a range of non-intended visitors (Wäckers *et al.* 2001). The latter applies especially to Lepidoptera, whose mouthpart morphologies restrict them to feeding on nectar with relatively low sugar concentrations. The shift from sucrose to hexose sugars may also reduce the attractiveness of extrafloral nectar to Lepidoptera (Wäckers *et al.* 2007).

Pattern of Extrafloral Nectar Production and (Risk of) Herbivory.

Most plants produce some level of extrafloral nectar irrespective of the presence of herbivores. However, even in undamaged plants this constitutive nectar production may be synchronized with the general periods of activity of damaging herbivores (Tilman 1978) and with the most susceptible stages of plant growth (Bentley 1977).

The constitutive nectar production may provide a degree of prophylactic protection, because it allows plants to accommodate some natural enemies before herbivores arrive (Wäckers *et al.* 2001). Prophylactic protection may be generated when predators interfere with herbivore oviposition or when they remove herbivore eggs. Maintaining some baseline level of ant visitation will also expedite the recruitment of additional ants in case of herbivore attack.

In addition to the constitutive production of food supplements, plants have been shown to actively adjust their food provision in response to their biotic environment. Unlike other defense mechanisms, this induction can be elicited by two distinct mechanisms. Nectar secretion can be raised both in reaction to tissue damage (Heil *et al.* 2001; Wäckers & Bezemer 2003; Wäckers *et al.* 2001) and in reaction to food

removal (Koptur 1992). These mechanisms represent active responses by the plants to both ant attendance and herbivore feeding. This receptiveness toward the presence of both the second and the third trophic level represents a unique and highly dynamic type of plant response. Besides raising nectar production from existing nectaries, herbivory can also result in increased formation of extrafloral nectaries on new growth (Mondor & Addicot 2003).

In addition to the temporal pattern of defense induction in response to herbivory, plant responses often also show a distinct spatial pattern. Extrafloral nectar production is especially suited to the study of spatial defense dynamics. Extrafloral nectar production can be easily assessed because of the discrete distribution of nectaries, the possibility of non-destructive sampling, as well as the ease of nectar collection. With respect to the spatial pattern of induction, (Wäckers *et al.* 2001) showed that foliar herbivory in cotton results in a largely localized induction of foliar nectar production, with the bulk of nectar being secreted from the damaged leaf. This local increase in nectar production can help in actively guiding ants to the site of attack. In addition, a weaker systemic response was found in younger leaves. In sharp contrast, root herbivory results in a plant-wide increase in nectar production (Wäckers & Bezemer 2003) (Fig. 1). Moreover, the pattern of extrafloral nectar production is distinctly different from the pattern in which secondary metabolites (terpenoids) are induced (Bezemer *et al.* 2003).

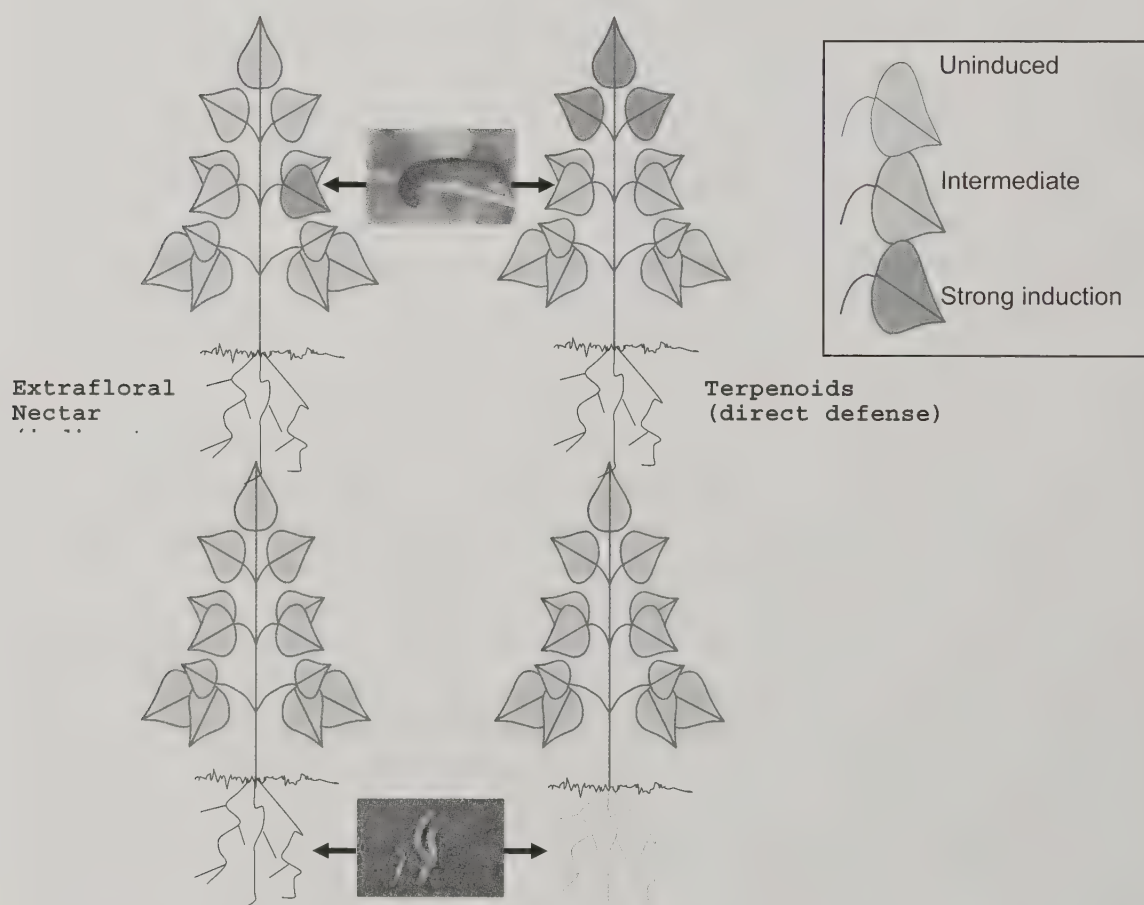


Fig. 1. Pattern of extrafloral nectar- and terpenoid-induction following tissue damage by root or shoot feeding herbivores. Based on Wäckers & Bezemer (2003) and Bezemer *et al.* (2003).

Extrafloral Nectar Production and the Value of Plant Tissue.

Vegetative tissues are usually less valuable and relatively easy to replace, whereas reproductive structures (flowers and particularly fruits) represent a high value due to their direct link to the plant's reproductive success. Moreover, reproductive structures are often more likely to be attacked (Zangerl & Bazzaz 1992) and even low levels of damage may result in fruits being aborted (Karban & Baldwin 1997). Based on these differences, the ODT makes two predictions with respect to defense allocation between vegetative and reproductive structures: (1) reproductive structures should receive a higher proportion of the overall defensive investment, and (2) foliar defenses should be inducible, whereas reproductive tissues are predicted to have high levels of constitutive (i.e. non-inducible) defense (Zangerl & Bazzaz 1992). Using *G. hirsutum*, a species that features both foliar and bracteal extrafloral nectaries, we tested whether nectar production patterns fit these ODT predictions. With regard to the first prediction, we were able to show that bracteal nectar secretion greatly exceeds foliar nectar production. Secondly, whereas foliar nectar production is clearly inducible, bracteal nectar production is not raised following herbivory (Wäckers & Bonifay 2004). The high level of constitutive bracteal nectar production likely provides 'prophylactic' protection for the valuable and vulnerable fruits. The less valuable leaves are protected in a 'curative' manner with low constitutive production and a sharp increase in nectar secretion following herbivory. This demonstrates that an inducible and a non-inducible variant of an indirect defense can occur together within one plant species.

CONCLUSIONS.

In this paper we test the defensive function of cotton extrafloral nectar by examining whether the temporal and spatial pattern in which cotton species allocate extrafloral nectar reduces defensive costs and/or increases defensive benefits. The broad agreement between optimal defense theory predictions and actual allocation patterns lends further support to the defensive function of cotton extrafloral nectaries.

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ATTRACT AND REWARD: A NOVEL APPROACH TO ENHANCE BIOLOGICAL CONTROL

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ABSTRACT.

Conservation biological control can be improved by combining two 'eco-technologies' to enhance the ability of natural enemies to reduce pest populations. In the 'attract and reward' approach, synthetic herbivore induced plant volatiles (HIPVs) (attract) are combined with floral resource supplementation (reward). The aim is to go beyond orthodox conservation biological control which, by providing floral resources, may only re-distribute natural enemies within the crop. By using HIPVs to attract enemies from surrounding habitats and by providing flowers enemies' ecological fitness and efficacy are likely to be improved. This work determined whether the use of synthetic methyl salicylate (MeSA) causes any increase in the abundance of arthropods over three trophic levels in turnip, *Brassica rapa* (L.) (Brassicaceae). A randomised block design with 12 replicates and two treatments (MeSA + yellow sticky trap and yellow sticky trap alone) were used. 'Target' species were *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) at the third trophic level, *Scaptomyza flava* (Fallén) (Diptera: Drosophilidae) at the second trophic level and *Anacharis zealandica* Ashmead (Hymenoptera: Figitidae) at the fourth trophic level. Contrary to the expectation that MeSA would attract only natural enemies, the results show a significant increase in abundance of species over three trophic levels. MeSA has a positive effect on the abundance of natural enemies within a crop. However, when deploying such attractants to improve biological control, the consequences of increased abundance of other arthropods in the insect community need to be taken into consideration.

DIFFERENTIAL EFFECTS OF THE INDUCTION OF DIRECT PLANT DEFENCES ON THE DEVELOPMENT OF GENERALIST AND SPECIALIST HERBIVORES AND THEIR SOLITARY ENDOPARASITOIDS

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ABSTRACT.

Amongst various types of plant defences that have been described, allelochemicals have been shown to play an important role against insect herbivory. However, domestication has often altered certain phenotypic plant traits, such as the size of vegetative structures or taste. For instance, cultivated plants often contain significantly lower levels of allelochemicals than their wild relatives. The aim of the present study is to evaluate host-plant quality in different wild and cultivated populations of wild cabbage, *Brassica oleraceae* L. (Brassicaceae), for two insect herbivores, a specialist and a generalist. Wild cabbage grows along the rocky coastlines of Britain and northern France, and there is considerable genetic variation in the expression of their defence compounds (glucosinolates) over limited spatial scales. To examine whether higher trophic levels are affected by the quality of the host plant, performance of two larval endoparasitoid species that differ in host specialization was compared as well. Glucosinolate concentrations in leaf tissue were measured as an indicator of plant defence. We examined the effect of the different food plants on the performance (survival, body mass development time) of the generalist herbivore, *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae) and its larval endoparasitoid, *Microplitis mediator* Haliday (Hymenoptera: Braconidae), as well as on the specialist herbivore, *Pieris rapae* (L.) (Lepidoptera: Pieridae) and its larval endoparasitoid, *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae). The results reveal that the performance of *M. brassicae* is strongly negatively correlated with total glucosinolate levels, whereas in *P. rapae* performance is more adversely related to the production of indole glucosinolates than total glucosinolate levels. Parasitoid performance in both species tracks that of the host.

SESSION 14

FOOD WEB INTERACTIONS AND IMPACT ON BIOLOGICAL CONTROL

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ABSTRACT.

The growing adoption of organic agriculture and related sustainable agricultural practices is encouraging greater biodiversity on farms. But is greater on-farm biodiversity a good thing? Only recently have studies begun to explicitly examine the contribution of predator and prey species richness to pest control. Already, it is clear that all possible results are found in various systems: in some cases herbivore control improves with growing diversity, but in other cases herbivore control is unchanged or even weakens as biodiversity increases. Disrupted pest control at higher diversity levels is often attributed to intraguild predation or predators switching to attack non-pest prey. However, a more complex array of mechanisms can lead to positive diversity effects, including predator-predator complementarity and facilitation, and apparent competition between pest and non-pest prey. The idiosyncrasy of these diversity effects presents a challenge for biocontrol workers, who seek to harness any advantages of biodiversity for pest control, while avoiding inadvertently worsening pest problems. What is needed is a more general, mechanistic understanding of the relationship between community diversity and herbivore suppression. The central goal of our symposium is to contribute to a greater understanding of when, and how, greater community complexity improves biological control (or disrupts it). Finke and Snyder will present the results of a series of experimental manipulations of natural enemy biodiversity, and will discuss mechanisms underlying stronger pest suppression with greater predator diversity. Eubanks and Montserrat will discuss the bottom-up effects of prey diversity on predator community structure, and the role of induced plant defenses in mediating these effects. Finally, Walzer will address the role of predator avoidance behavior in mediating predator-predator interactions.

SCARED SICK? PREDATOR-PATHOGEN COMPLEMENTARITY STRENGTHENS BIOLOGICAL CONTROL

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ABSTRACT.

Resource exploitation generally intensifies with greater consumer biodiversity, implying an underlying resource partitioning that has been difficult to convincingly demonstrate. Pathogens and predators are two classes of consumer that often exhibit differences in ecologically important traits (e.g., size, resource acquisition strategy, foraging location) that could lead to complementary effects on shared prey/hosts. To examine this possibility, we manipulated species richness among a community of pathogens and predators attacking an herbivorous beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), and measured resulting effects on herbivore suppression. We found that herbivore mortality increased, and plant damage decreased, when more natural enemy species were present. However, closer examination revealed that it was the pairing of predator with pathogen species, rather than greater biodiversity *per se*, that strengthened herbivore suppression. In this community the predators occur aboveground and attack herbivore juvenile stages in plant foliage, whereas the pathogens occur belowground and attack herbivores pupating in the soil. In a subsequent field experiment we tracked the emergence of predator-pathogen complementarity throughout the course of beetle development. We found that herbivores exposed to predators aboveground were more susceptible to subsequent pathogen infection belowground, consistent with our observation in the laboratory that predator exposure weakens beetles' immune response. Thus, predators facilitated resource capture by pathogens, perhaps reflecting an inherent conflict for the herbivore in allocating energetic resources towards anti-predator versus anti-pathogen defenses. It seems that predator-pathogen pairings were particularly taxing not because these natural enemies partitioned resources among themselves, but rather because they enforced the partitioning of resources internal to prey/host individuals.

APHIDS AS BENEFICIAL INSECTS? THE INDIRECT EFFECTS OF APHIDS ON THE SUPPRESSION OF OTHER ARTHROPODS

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ABSTRACT.

We are studying the indirect effects of cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae) on arthropod pests of cotton, *Gossypium hirsutum* L. (Malvaceae) via aphid-induced changes in the abundance and behavior of predators and aphid-induced changes in plant chemistry. We have found that the indirect effects of aphids via these mechanisms on key pest and beneficial species are strong. Aphids attract mutualistic ants and aphid predators that have strong, negative effects on the abundance of non-aphid herbivores such as caterpillars and aphids induce the production of defensive compounds in cotton plants that negatively affect caterpillars. Caterpillars and other non-aphid pests are often far more economically important than cotton aphids to cotton production, thus indirect suppression of these herbivores by cotton aphids results in increased cotton yield under a wide range of conditions (e.g., moderate to high levels of other pests).

THREAT-SENSITIVE PREDATOR AVOIDANCE WITHIN A GUILD OF ACARINE BIOLOGICAL CONTROL AGENTS

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ABSTRACT.

The threat-sensitive predator avoidance hypothesis predicts that prey is able to assess the magnitude of predator threat and adjusts its behavior accordingly. This concept has been supported by graded anti-predation responses from several species in classical prey-predator interactions, but has not yet been tested for within trophic level interactions such as intraguild (IG) predation. The predatory mites *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus californicus* (McGregor) and *Amblyseius andersoni* Chant (Acari: Phytoseiidae) co-occur in natural settings and may be simultaneously used in biocontrol of herbivorous mites and insects. All three species prey on spider mites but differ greatly in diet specificity with *P. persimilis* being highly specialized, *N. californicus* being intermediate and *A. andersoni* being the least specialized. The ranking with regard to the propensity to IG is: *A. andersoni* > *N. californicus* > *P. persimilis*. Thus, the three species differ in the predation threat posed to each other. Since larvae are the most vulnerable and most often attacked IG prey, maternal strategies to reduce larval predation risk such as avoidance of oviposition in IG environments are expected to be selected for. Additionally, these maternal behaviors could be influenced by experience with IG predators. We tested these assumptions with naïve and IG-experienced females of *P. persimilis* given a choice between a prey patch with spider mites and a prey patch with spider mites and IG predator cues from either a high (*A. andersoni*) or low risk predator (*N. californicus*). Preliminary results are given and potential implications to biological control are discussed.

NICHE PARTITIONING INCREASES PREY SUPPRESSION BY DIVERSE ENEMY COMMUNITIES

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ABSTRACT.

Classical ecological theory suggests that consumer species coexistence is fostered by resource-use differences, leading to greater resource utilization in communities with more species. However, explicit empirical support is lacking because resource use by species generally is confounded with other species-specific attributes. We overcome this obstacle by co-opting behavioral plasticity in host choice among a group of parasitoid wasps, allowing us to manipulate patterns of resource use while controlling for the effects of species identity and diversity. We conducted this study using the community of parasitoids, *Aphidius colemani* Viereck, *Aphidius matricariae* Haliday, and *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae) which attacks three aphid species (*Brevicoryne brassicae* (L.), *Lipaphis erysimi* (Kaltenbach), and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) on radishes, *Raphanus sativus* L (Brassicaceae). Within this aphid-parasitoid-radish community, we created a fully-factorial manipulation of consumer resource-use breadth (specialist versus generalist) and species diversity (1 versus 3 species) and found that resource exploitation improves with greater specialist, but not generalist, diversity. Therefore, resource partitioning, and not diversity *per se*, fosters greater overall resource consumption in our multi-species consumer communities. The implication is that efficient biological control of pests would be best achieved by utilizing multiple enemy species which vary in their resource preferences.

ALTERNATIVE PREY IMPROVES THE COMBINED EFFECT OF AN OMNIVORE AND A PREDATOR ON BIOLOGICAL PEST CONTROL. A CASE STUDY IN AVOCADO ORCHARDS

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ABSTRACT.

Top-down control in three-trophic level food chains is the core concept in biological pest control. However, two factors complicate this simple way of picturing agricultural communities. First, agro-ecosystems are composed of several species that interact, forming complicated food webs. Second, the structure of agricultural communities may not be temporally stable. Efficient pest management approaches need to integrate these two factors to generate better predictions for pest control. In this work, we identified the food web components of an avocado agro-ecosystem, and unravelled through field and lab experiments when species co-occur and how they interact with each other. This allowed us to predict how the community could be modified to improve the performance of the naturally occurring predators. Predictions were further tested in field population experiments. Field surveys revealed that the structure and the species of the avocado community shifted through time. In spring, the community was characterized by a “non-herbivore” prey (pollen) and an omnivorous predatory mite, trophically linked linearly. In summer, the community was composed of an omnivore and a predator sharing an herbivore as prey. The link between omnivore and pollen disappeared, and potential competition or intraguild-predation between omnivore and predator arose. We artificially increased the temporal coexistence of the two communities via addition of the non-herbivore prey for the omnivore, to (1) lessen the potential intraguild predation between predator and omnivore, and (2) induce apparent competition between alternative prey and herbivore, mediated by the shared omnivore, resulting in (3) reduced populations of the herbivore.

SESSION 15

PROGRESS AND PROSPECTS TO ASSESS PREDATION

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ABSTRACT.

Predation remains a difficult process to quantify, particularly under field conditions. Traditional methods such as direct observation, field cage inclusion, or field cage exclusion are now complemented by a range of qualitative serological and molecular methods to detect prey remains within the gut contents of the predator. Development of detection methods is only the first step to understanding predation. The next step is to apply those methods in the field and to interpret the results – something that is often easier said than done, as our symposium will show. The speakers will discuss predation measured using a combination of ELISA, PCR and observational methods in cotton, coffee, corn and other agroecosystems. Both insect and arachnid predators are considered feeding on a wide variety of pests. From these presentations, we hope to demonstrate recent advances in methodology while identifying limitations on their application in the field. This should point the way to new questions and research areas relevant to particular agroecosystems and to the study of predation as a whole.

PINPOINTING PREDATION EVENTS: A DIFFERENT MOLECULAR APPROACH

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ABSTRACT.

A glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*, protein marking system has been developed as a diagnostic tool for quantifying predation rates via gut content analysis. A field study was conducted to quantify predation rates on each of the GWSS lifestages. Specifically, two GWSS nymphs and two adults were marked with either rabbit IgG, chicken IgG, milk protein, or soy protein and then released into field cages containing a known assemblage of predators. Additionally, a sentinel GWSS egg mass was placed in each cage. In turn, the stomach contents of every predator in each cage was examined by four different protein-specific ELISAs and a GWSS egg-specific ELISA to detect for the presence of the targeted prey items. Here we present the results obtained for two of the predators examined; the convergent lady beetle, *Hippodamia convergens* and the praying mantis, *Stagmomantis carolina*. ELISA results indicated that two of the nine praying mantids examined fed on a single GWSS adult while five and two of the 78 lady beetles fed on a single GWSS adult and nymph, respectively. There was no GWSS egg predation detected and none of the predators consumed multiple GWSS prey.

INTRODUCTION.

Predators can be important regulators of arthropod populations (Luff 1983). However, accurately identifying key predators of most pests is difficult because predators and their prey are often small, elusive, and cryptic. Hence, visual field observations of predation are extraordinarily difficult to obtain. Perhaps the most frequently used experimental approach for evaluating predaceous natural enemies in the field is the cage study (Luck *et al.* 1988). Such studies require manipulation of either the natural enemy or the targeted prey population(s) within the cage. Pest mortality can be estimated based on the presence or absence of the pest over time (Smith & De Bach 1942; Luck *et al.* 1988). Such studies have documented the qualitative impact of manipulated predator assemblages on many types of pests, but they do not provide quantitative information on predation rates or evidence of which predator in the assemblage is exerting the greatest biological control. Often the only direct evidence of arthropod predation can be found in the stomach contents of predators. Currently, the state-of-the-art predator stomach content assays include immunoassays (typically ELISA) for the detection of pest-specific proteins (Hagler & Naranjo 1996) and PCR assays for the detection of pest-specific DNA (de León *et al.* 2006).

ELISAs using pest-specific monoclonal antibodies (MAbs) have been widely used to identify key predators of certain pests, including the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae) (Fournier *et al.* 2006). The simplicity and low cost of ELISA lends itself to the efficient screening of hundreds of field-collected predators per day (Hagler & Naranjo 2005). However, MAb development is too technically difficult, costly, and time consuming for wide scale appeal (Greenstone 1996). Moreover, pest-specific ELISAs share the same limitation as the other predator evaluation methods; the quantification of predation rates is impossible (reviewed by Hagler & Naranjo 1996). PCR assays using pest-specific DNA probes might be less expensive to develop (Greenstone & Shufran 2003), but PCR assays are also not quantifiable and they are more costly, technical, tedious, and time consuming than ELISAs (Fournier *et al.* 2008). These difficulties have resulted in a dearth of information on the quantitative impact that generalist predators have on suppressing pest populations.

The many shortcomings of each method of predator assessment described above were the impetus for us to develop a more efficient screening technique for predator activity. Our goal is to: **(1)** quantify predation rates on GWSS nymphs and adults and **(2)** qualify predation on GWSS eggs. Using a multiple prey marking technique (Hagler 2006) and a GWSS egg-specific MAb (Fournier *et al.* 2006) we simultaneously examined the gut contents of predators for the presence of five GWSS prey items (e.g., GWSS egg protein, two protein marked nymphs and two protein marked adults). Here, we provide a summary of the gut content analyses for two of the predator species examined.

MATERIALS AND METHODS.

Laboratory Study.

The first experiment was conducted to determine if protein markers can be substituted for pest-specific MAbs in the immunological detection of prey in predator guts. GWSSs were not used in this study because the feeding study was conducted in an area quarantined for the GWSS (Phoenix, Arizona). Instead, we selected predators and prey for the feeding trials that represent extreme-case scenarios for detecting predation using molecular gut content assays. The “easy-case” scenario was a striped earwig, *Labidura riparia* (Pallas) (Dermaptera: Labiduridae), a large chewing predator, feeding on a large protein marked pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) larva. The “tough-case” scenario was a minute pirate bug, *Orius tristicolor* (White) (Heteroptera: Anthocoridae), a small piercing and sucking predator, feeding on a very small protein marked parasitoid, *Eretmocerus* sp. (Hymenoptera: Aphelinidae). In a series of lab studies, we fed these predators prey items marked with rabbit immunoglobulin G (IgG). In turn, the gut contents of each predator was analyzed by a rabbit IgG-specific ELISA to detect for the presence of rabbit IgG in their gut. Details of the methods used are given in Hagler (2006).

Field Study.

Field studies were initiated to quantify predation rates on GWSS nymphs and adults using multiple protein markers and qualify predation on GWSS eggs using a GWSS-specific sandwich ELISA. Details of the experimental design are provided by Hagler (2006) and Hagler *et al.* (submitted). Briefly, we erected 40, 1-m long field cages on selected citrus branches. We then placed (using a paper clip) a single sentinel GWSS egg mass containing 6 to 12 eggs per mass on the underside of a randomly selected leaf in each cage along with two individuals each of the convergent lady beetle, *Hippodamia convergens* Guérin Méneville (Coleoptera: Coccinellidae), *Collops vittatus* Say (Coleoptera: Melyridae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *L. riparia*, *Geocoris punctipes* Say (Heteroptera: Lygaeidae); and one individual each of *Sinea confusa* Caudell (Heteroptera: Reduviidae) and *Zelus renardii* Kolenati (Heteroptera: Reduviidae). Moreover, a single praying mantis, *Stagmomantis carolina* (Johannson) (Mantodea: Mantidae), was placed in nine of the cages. One hour later, we released two uniquely marked GWSS adults and two uniquely marked nymphs into each of the 40 cages. The GWSS nymphs were marked with either non-fat dry milk or chicken IgG protein and the adults were marked with either rabbit IgG or soy milk protein.

After 6 h, each citrus branch was cut at its base, just below each cage, and immediately frozen on dry ice. Each predator was then analyzed by four protein-specific ELISAs to determine if they contained marked GWSSs in their guts. Additionally, the gut contents of each predator was examined by a GWSS egg-specific sandwich ELISA (Fournier *et al.* 2006) to determine the frequency of predation on GWSS eggs.

RESULTS.

Laboratory Study.

The feeding studies showed that, regardless of the predator species and the size of protein-marked prey consumed, that the prey marking ELISAs can easily detect the mark in the predator's stomach for at least 12 h after feeding (Table 1; Hagler 2006). These results suggest that this marking technique will be effective for identifying key predators of marked GWSSs.

Field Study.

All nine of the praying mantids were recovered from the field cages after the 6 h exposure period. The multiple gut content ELISA results for these nine individuals are presented in Fig. 1A. The results showed a strong positive response by individual 4 and 8 to the rabbit IgG ELISA. These data indicate that these two individuals consumed the rabbit IgG marked GWSS adult released into their respective cages. There was no evidence of predation on the GWSS nymph or egg lifestages.

Eighty lady beetles were released into the 40 field cages containing the marked GWSS prey items (2 per cage). Of these, 78 were recovered after the 6 h exposure period. The fate of the two missing beetles is unknown, but is likely due to escape

from the cages, intraguild predation, or being overlooked in the sorting process. The multiple gut content ELISA results for the 78 individuals recaptured after the 6 h study interval are presented in Fig. 1B. The ELISA results indicate that individual 7, 15, 38, 51, and 68 fed on a single GWSS adult (indicated by a positive soy or rabbit IgG ELISA reaction) and individual 60 and 76 fed on a single GWSS nymph (indicated by a positive milk or chicken egg white ELISA reaction); respectively. There was no evidence that any of these individuals fed on more than one GWSS. Moreover, there was no evidence that the beetles fed on GWSS eggs.

Table 1. Mean (\pm SD) ELISA readings for the retention of rabbit IgG in the gut of two types of predators that consumed either a single 2nd instar pink bollworm larva or an adult parasitoid (*Eretmocerus emiratus*) marked with 5.0 mg/ml of rabbit IgG (from Hagler 2006).

Predator	Hours After Feeding	n	Mean (\pm SD)	Percent ¹³ Positive
Earwig ^{1/}	Negative Control	30	0.07 (0.02)	0
	0	30	1.29 (0.66)	100
	24	30	1.39 (0.58)	100
	48	30	1.16 (0.92)	100
Minute Pirate Bug ^{2/}	Negative Control	32	0.04 (0.02)	0
	0	10	0.15 (0.10)	70.0
	3	10	0.07 (0.02)	70.0
	6	31	0.35 (0.31)	90.3
	9	10	0.17 (0.11)	80.0
	12	31	0.13 (0.13)	87.1

^{1/} Earwig was fed a single rabbit-IgG marked pink bollworm larva.
^{2/} Minute pirate bug was fed a single rabbit IgG-marked parasitoid.
^{3/} Percentage of predators scoring positive by the rabbit IgG ELISA.

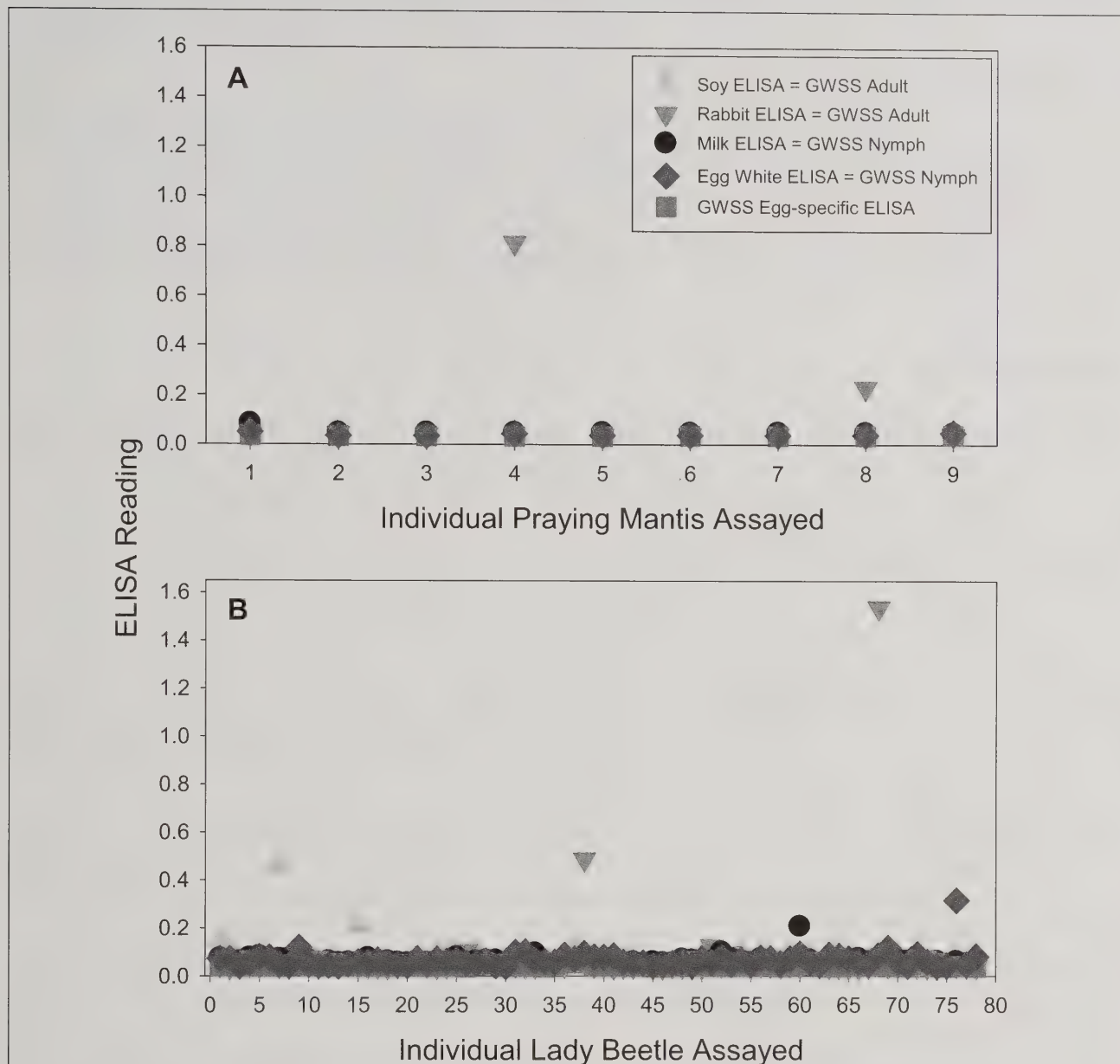


Fig. 1. ELISA results for every praying mantid (*Stagmomantis carolina*) and Convergent lady beetle (*Hippodamia convergens*) assayed for remains of differentially marked GWSSs.

DISCUSSION.

Although it is widely accepted that predators play a role in pest regulation, we still have an inadequate understanding of and ability to predict their impact in cropping systems. The impact that predators have on suppressing GWSS populations goes unrecognized due to the difficulties of assessing arthropod predation. The prey marking technique (Hagler 2006) combined with a GWSS egg-specific gut content ELISA (Fournier *et al.* 2006) circumvented many of the shortcomings of the current methods used to study predation. Here, we quantified predation on GWSS nymphs and adults by two predator species. Since these predation events were each detected with a specific protein ELISA, we are confident

that these results represent the first quantified results of predation using molecular gut content methods (e.g., immunological or DNA based).

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USING EXISTING METHODS IN NEW ECOSYSTEMS – CAN WE JUST PLUG AND PLAY?

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ABSTRACT.

A comparison of the insect fauna associated with cotton fields in the USA and Australia shows that there are many ecologically similar pest and beneficial species. Pest management strategies in both regions have followed a similar pattern of pest control with a shift from insecticide intensive regimes to an IPM system that incorporates conservation biological control with the use of selective insecticides. However, knowledge of the beneficial predator complex in Australian cotton lags behind that of USA cotton. The use of ELISA techniques to identify predators of particular pests began almost 20 years ago in the USA but use of such methods in Australian is much more recent. In this review we compare the effectiveness of ELISA techniques for several ecologically similar species. ELISA detection of prey marked with exotic proteins proved effective in both the original (USA) and new (Australia) ecosystems, but ELISA using a pest specific antibody was less effective in the new ecosystem.

INTRODUCTION.

Pest management guidelines for cotton in the USA and Australia have shifted from insecticide intensive strategies to IPM systems (Deutscher *et al.* 2005; UCIPM 2008). One factor that encouraged this shift in pest management is the introduction of genetically modified Bt cotton varieties that provide an alternative to conventional insecticides for control of lepidopteran pests (Fitt 2000). The development of insecticide resistance in key pests has also contributed to the uptake of alternative strategies (Ellsworth & Martinez-Carrillo 2001). The similarities between USA and Australian cotton systems are not confined to pest management – there are noticeable parallels between the pest and beneficial fauna in both places (Hearn & Fitt 1992). The boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) was an obvious exception prior to its eradication from most cotton growing regions in the USA, because it has never reached Australia (Hearn & Fitt 1992, USDA 2003). The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is also an exception because eradication efforts in Australia have confined the species to a limited geographic range (QDPI 2008).

Development of effective conservation biological control for cotton IPM requires an understanding of predator biology, particularly in relation to prey choice. This information is often hard to acquire outside of the laboratory because of the (now well recognised) difficulties in observing and quantifying predation under natural conditions. Gut content assays using ELISA are one method for identifying which predators feed on which prey. Both prey-specific monoclonal antibodies and exotic

protein markers have been used to detect recent predation. Development of these methods to study predators in USA cotton crops began almost 20 years ago (Hagler *et al.* 1991), targeting several prey and predator species (e.g. Hagler & Naranjo 1994a, b; Fournier *et al.* 2006). The use of ELISA to understand predator biology in Australian cotton systems is much more recent and has been confined to one target prey, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and several predators (Mansfield 2004; Mansfield *et al.* 2008). In this paper we summarise the results gained from ELISA methods applied to three pairs of similar predators from USA and Australian cotton systems (Table 1) to detect predation on the lepidopteran pests *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) and *H. armigera*. The effects of meal size, time elapsed since feeding and predator biology on the reliability of different ELISA methods is discussed in relation to these results.

Table 1. Species chosen for comparison of ELISA methods used to detect predation in USA and Australian cotton systems.

Order	Family	USA	Australia
Coleoptera	Melyridae	<i>Collops vittatus</i> (Say)	<i>Dicranolaius bellulus</i> (Guerin-Meneville)
	Coccinellidae	<i>Hippodamia convergens</i> Guerin-Meneville	<i>Hippodamia variegata</i> (Goeze)
Hemiptera	Geocoridae	<i>Geocoris punctipes</i> (Say)	<i>Geocoris lubra</i> Kirkaldy

MATERIALS & METHODS.

To develop and test the ELISA protocols under controlled conditions predators were collected from crops near to the authors' research stations in Arizona and New South Wales. Prey were supplied from laboratory cultures maintained on site. For all predators an indirect ELISA was used with prey-specific monoclonal antibodies (MAb) to detect predation on eggs of *P. gossypiella* and *H. armigera*. The MAb specific to *P. gossypiella* was developed by Hagler *et al.* 1994 and the MAb specific to *H. armigera* was developed by Trowell *et al.* 2000. A sandwich ELISA was used to detect predation on prey marked with the exotic protein, rabbit IgG (product no. R2004, Sigma-Aldrich). Details of predator collections, prey sources, ELISA protocols and prey marking procedures have been published previously (as listed in Tables 2-4).

Predation on *P. gossypiella* and *H. armigera* under natural conditions was investigated using prey-specific indirect ELISA. Predators were collected from cotton crops using vacuum sampling (Arizona, Hagler & Naranjo 1994a, b) and beat sheets (New South Wales, Mansfield *et al.* 2006).

RESULTS & DISCUSSION.

For the melyrid and coccinellid predators the anti-rabbit IgG ELISA generally performed better than the pest-specific ELISA (Tables 2 and 3). Predation was

detected for at least four hours after the melyrid beetles and *H. variegata* had consumed a large meal (> 5 eggs). Even small meals (1 egg) were detected in *H. convergens* for up to 24 hours after feeding but detection intervals for large meals are not known for this species. It was possible to detect predation by *D. bellulus* using the anti-*H. armigera* ELISA although there was a high probability of a false negative when the meal size was small (1-2 eggs) compared with larger meals (> 5 eggs). Detection of predation by *C. vittatus* using the anti-*P. gossypiella* ELISA was likely even when the meal size was small but there is no information available regarding the effect of digestion time. The effect of meal size on the anti-*H. armigera* ELISA was greater for *H. variegata* than *D. bellulus* because small meals were not detected in *H. variegata* even immediately after consumption. The pest-specific ELISA was slightly more effective for *H. convergens* than *H. variegata* because small meals were detectable for a brief period after consumption by *H. convergens* even if the rate of false negatives was high (> 70%). For both coccinellids large meals were unlikely to be detected with a pest-specific ELISA once two hours had elapsed. This difference in the performance of the pest-specific indirect ELISA and anti-rabbit IgG sandwich ELISA when applied to predatory beetles can be partly attributed to the higher total protein content of these insects. This affects the sensitivity of the indirect ELISA leading to a high probability of false negatives (Hagler *et al.* 1997).

Table 2. Detection times for recent egg predation by *Collops vittatus* on *Pectinophora gossypiella* and *Dicranolaius bellulus* on *Helicoverpa armigera*.

Predator	Prey	ELISA ¹	Meal size (eggs)	Time after feeding (h)	Sample size ²	Positive (%)	Reference
<i>C. vittatus</i>	<i>P. gossypiella</i>	MAb	1-2	1	10	90	Hagler <i>et al.</i> 1994
		Rabbit	1	0	10	100	Hagler& Durand 1994
			1	1	30	100	
			1	2	10	90	
			1	4	10	100	
			1	6	10	30	
			1	12	10	20	
			1	24	10	20	
			1	48	10	10	
			1	72	10	10	
<i>D. bellulus</i>	<i>H. armigera</i>	MAb	5-10	0	26	88	Mansfield <i>et al.</i> 2008
			5-10	1	18	94	
			5-10	2	18	94	
			5-10	4	17	71	
			5-10	24	15	0	
			1	0	158	40	Mansfield 2004 ³
			1	1.8		20	
			1	3.5		10	
		Rabbit	5-10	0	26	100	Mansfield <i>et al.</i> 2008
			5-10	1	18	100	
			5-10	2	18	100	
			5-10	4	17	100	
			5-10	24	15	40	

¹ Assay methods were an indirect prey-specific ELISA (MAb) and a sandwich ELISA to detect prey marked with rabbit IgG (Rabbit).

² Number of individuals assayed.

³ Detection times calculated from an exponential decay curve fitted to raw data. Sample size is total number of individuals assayed to produce the curve.

From the limited data available, the pest-specific ELISA may be more effective than the anti-rabbit IgG for detecting egg predation by the bigeyed bugs, *Geocoris* spp. Prey remained detectable in *G. punctipes* for a much longer period (> 5 h for large meals, Table 4) after consumption using the anti-*P. gossypiella* ELISA compared with the same ELISA used on *C. vittatus* and *H. convergens*. The detection interval was still short (approximately 2 h for a small meal) when the anti-*H. armigera* ELISA was applied to *G. lubra* but the probability of detection within that time was greater than it was for *D. bellulus* and *H. variegata*. The anti-rabbit IgG ELISA has not been used to detect predation on *H. armigera* by *G. lubra*. This difference in the performance of the pest-specific indirect ELISA and anti-rabbit IgG sandwich ELISA when applied to *Geocoris* spp. is probably due to their mode of feeding – sucking predators tend to take up smaller quantities of rabbit IgG than do chewing predators (Hagler & Durand 1994). However prey marking has been used successfully to detect predation by other Hemiptera (Reduviidae and Nabidae, Hagler 2006).

Table 3. Detection times for recent egg predation by *Hippodamia convergens* on *Pectinophora gossypiella* and *H. variegata* on *Helicoverpa armigera*.

Predator	Prey	ELISA ¹	Meal size (eggs)	Time after feeding (h)	Sample size ²	Positive (%)	Reference
<i>H. convergens</i>	<i>P. gossypiella</i>	MAb	10	0.8	75	75	Hagler & Naranjo 1997
			10	2.3	75	50	
			10	5	75	25	
			6	0.2	75	75	
			6	1.3	75	50	
			6	3.2	75	25	Hagler 1998
			1	0	15	27	
			1	1	15	13	
		Rabbit	1	0	10	100	Hagler & Durand 1994
			1	1	30	100	
			1	2	10	100	
			1	4	10	100	
			1	6	10	100	
<i>H. variegata</i>	<i>H. armigera</i>	MAb	1	12	10	70	Mansfield et al. 2008
			1	24	10	90	
			1	48	10	0	
			5-10	0	18	83	
			5-10	1	18	67	
			5-10	2	16	13	Mansfield 2004
			5-10	4	16	0	
			5-10	24	15	0	
			1	0	17	0	
		Rabbit	5-10	0	18	100	Mansfield et al. 2008
			5-10	1	18	100	
			5-10	2	16	100	
			5-10	4	16	100	
			5-10	24	15	100	

¹Assay methods were an indirect prey-specific ELISA (MAb) and a sandwich ELISA to detect prey marked with rabbit IgG (Rabbit).

²Number of individuals assayed.

How should differences in observed predation rates between different types of field experiments or surveys be interpreted? For example, the proportion of field collected *C. vittatus*, *H. convergens* and *G. punctipes* that tested positive for predation on natural populations of *P. gossypiella* (using the anti-*P. gossypiella* ELISA, Table 5) is comparable to results with the same ELISA from a recent field cage study where both prey and predators were introduced deliberately into the cages (Hagler 2006). Yet this was not the case for two of the three predator species when results of field surveys (using the anti-*H. armigera* ELISA, Table 5) and a study that used sentinel eggs were compared (Mansfield et al. 2008). More *D. bellulus* tested positive (18%) for predation on sentinel *H. armigera* eggs than for predation on natural populations of *H. armigera* (< 1%). No *G. lubra* tested positive for predation on sentinel *H. armigera* eggs but nearly 8% tested positive for predation on natural populations. No predation by *H. variegata* was detected using the anti-*H. armigera* ELISA in either situation.

Table 4. Detection times for recent egg predation by *Geocoris punctipes* on *Pectinophora gossypiella* and *G. lubra* on *Helicoverpa armigera*.

Predator	Prey	ELISA ¹	Meal size (eggs)	Time after feeding (h)	Sample size ²	Positive (%)	Reference
<i>G. punctipes</i>	<i>P. gossypiella</i>	MAb	10	6.2	142	75	Hagler & Naranjo 1997 ³
			10	15.3		50	
			10	30.8		25	
			6	3.9	157	75	
			6	9.8		50	
			6	20.9		25	
			3	1.5	143	75	
			3	5.5		50	
			3	12.4		25	
			1	0.9	132	50	
			1	4.1		25	
			1-2	1	10	60	Hagler et al. 1994
		Rabbit	1	1	20	30	
<i>G. lubra</i>	<i>H. armigera</i>	MAb	1	0	80	75	Mansfield 2004 ³
			1	0.75		50	
			1	2.1		25	

¹Assay methods were an indirect prey-specific ELISA (MAb) and a sandwich ELISA to detect prey marked with rabbit IgG (Rabbit).

²Number of individuals assayed.

³Detection times calculated from an exponential decay curve fitted to raw data. Sample size is total number of individuals assayed to produce the curve.

CONCLUSIONS.

The ability of either prey-specific or exotic protein ELISA techniques to detect predation under natural conditions is affected not only by the factors discussed above (meal size, decay rates of the antigen, predator species) but also by the interaction between the target prey, predator and ELISA method. The short detection interval and high rate of false negatives for the anti- *H. armigera* ELISA limited its usefulness for detecting predation under natural conditions compared with the anti- *P.*

gossypiella ELISA. The anti-rabbit IgG ELISA has a longer detection interval and lower rate of false positives but has only been used in one limited field study in Australia. Protein marking has been used extensively in USA cotton but the target prey have been mostly Hemiptera not Lepidoptera. Here we have considered only lepidopteran prey because ELISA methods have not been used to detect predation on any other prey in Australian cotton systems. Further comparisons between USA and Australian cotton systems may prove useful in future if ELISA methods are used to study predation on hemipteran pests in Australian cotton.

Table 5. Field collected predators that tested positive (%) for recent predation on eggs of *Pectinophora gossypiella* and *Helicoverpa armigera* using pest-specific MAb and indirect ELISA.

Prey	Predator	Sample size	Positive (%)	Reference
<i>P. gossypiella</i>	<i>C. vittatus</i>	680	14.0	Hagler & Naranjo 1994a, b
	<i>H. convergens</i>	614	3.6	
	<i>G. punctipes</i>	615	3.6	
<i>H. armigera</i>	<i>D. bellulus</i>	2979	0.9	Mansfield 2004
	<i>H. variegata</i>	132	0	
	<i>G. lubra</i>	104	7.7	

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BIOLOGICAL CONTROL OF COFFEE BERRY BORER: THE ROLE OF DNA-BASED GUT-CONTENT ANALYSIS IN ASSESSMENT OF PREDATION

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ABSTRACT.

The coffee berry borer, *Hypothenemus hampei*, is the most important pest of coffee worldwide, causing an estimated \$500 million in damage annually. Infestation rates from 50-90% have been reported, significantly impacting coffee yields. Adult female *H. hampei* bore into the berry and lay eggs whose larvae hatch and spend their entire larval life within the berry, feeding on the coffee bean, lowering its quality and sometimes causing abscission. Biological control of *H. hampei* using parasitoids, fungi and nematodes has been reported but potential predators such as ants and predatory thrips, which have been observed in and around the coffee berries, have received little attention. This study reviews previous *H. hampei* biological control efforts and focuses on the role of predators in *H. hampei* biological control, an area in which tracking trophic associations by direct observation is not possible in part due to the cryptic nature of the biology of *H. hampei*, spending its life cycle inside the berry. The use of molecular methods to detect the presence of small amounts of prey in the digestive tracts of predators is the primary focus of this research program, and ultimately elucidating food web structure and making recommendations for biological control. We designed *H. hampei*-specific primers to demonstrate that *H. hampei* DNA can be detected in DNA extractions of a predatory thrips species, *Karnyothrips flavipes*, which preys on *H. hampei*. We demonstrate the potential of this molecular technique to unravel the trophic interactions that occur inside the coffee berry.

INTRODUCTION.

Coffee (Rubiaceae: *Coffea* spp.) is the most important agricultural commodity in over 70 countries, accounting for over US \$70 billion in annual retail value (Vega *et al.* 2006). Of the more than 100 species in the genus *Coffea* (Davis *et al.* 2006), only two species, *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner are grown commercially. Small-scale farmers produce ~70% of the world's coffee, with over 100 million people depending on its production (Vega *et al.* 2003). Coffee is attacked by more than 850 species of insects (Le Pelley 1968, 1973). Of these, the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae), is the only species that directly attacks the seed (Vega *et al.* 2003), and is the main threat to coffee production (Damon 2000; Jaramillo *et al.* 2006), with annual losses

exceeding US \$500 million (Vega 2004). This pest was first described in 1867 in France feeding on coffee beans (Waterhouse & Norris 1989) and has subsequently spread to all coffee producing countries except Hawaii (Vega 2004) and Nepal. In this paper, we review the biology and ecology of *H. hampei*, examine various biological control efforts, and demonstrate the potential for DNA-based detection methods in unraveling trophic interactions between these cryptic pests and their natural enemies.

BIOLOGY AND ECOLOGY OF *HYPOTHENEMUS HAMPEI*.

The life cycle of *Hypothenemus hampei*.

Developing coffee berries are typically attacked by single mated female *H. hampei* from between eight weeks after flowering until harvest (>32 weeks) (Baker 1999). It takes up to eight hours for adult female *H. hampei* to bore through a coffee berry to reach the endosperm (Sponagel 1994; Fig. 1). A female *H. hampei* lays 200-300 eggs over a period of 60 days (Jaramillo 2008). At 27°C, the egg stage averages 4.3 days, the three larval stages average a total of 12.0 days, and the pupal stage 5.2 days (Jaramillo 2008). Due to the 60-day oviposition period, all life stages co-occur in the berry. Mating occurs between siblings within the berry (Bustillo *et al.* 1998), after which the female either remains in the berry to commence oviposition, or exits in search of another berry to colonize (Baker *et al.* 1992). High temperature and relative humidity triggers emergence of mated females (Baker *et al.* 1992) but males spend their entire life cycle inside the berry (Ticheler 1961). During the interharvest period, a single berry may contain as many as 150 adult *H. hampei*, and reproduction only ceases once all resources have been consumed (CENICAFÉ 1993).

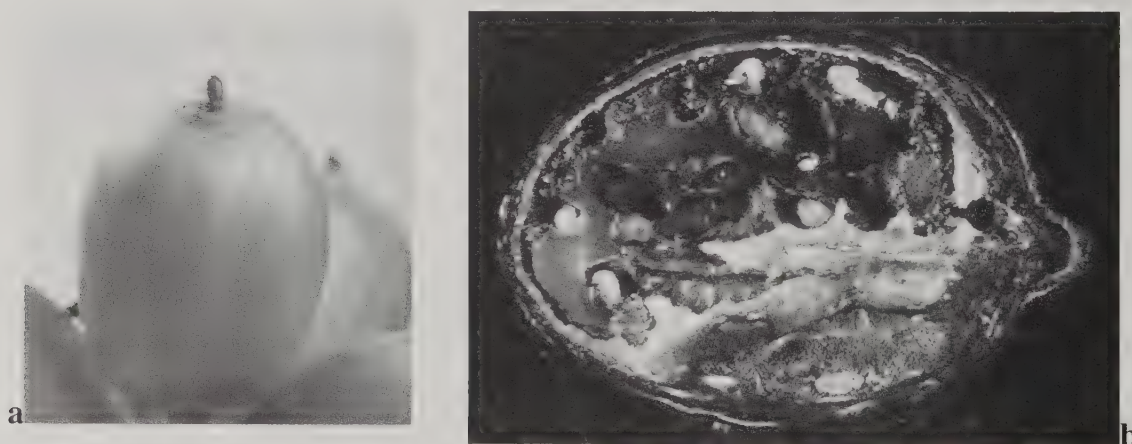


Fig. 1. (a) Female *Hypothenemus hampei* penetrating a coffee berry (b) *Hypothenemus hampei* life stages inside a coffee berry.
(Photographic acknowledgment: G. Hoyos CENICAFÉ, Chinchiná, Colombia).

Damage caused by *Hypothenemus hampei*.

Hypothenemus hampei causes three types of damage. First, because they feed on the endosperm, there are both losses in overall yield and in quality of the beans (Le Pelley 1968; Moore & Prior 1988). Secondly, mature berries become vulnerable to

further insect attack and infection by fungi due to the physical damage caused by the boring and feeding activities of *H. hampei* (Waterhouse & Norris 1989). Thirdly, *H. hampei* infestations can result in arrested development, decay or premature fall of the berry (Le Pelley 1968).

BIOLOGICAL CONTROL OF *HYPOTHENEMUS HAMPEI*.

Species of parasitic and predatory Hymenoptera and entomopathogenic nematodes and fungi, have all been examined to various degrees for their potential role as biological control agents of *H. hampei*.

Parasitoids.

Three species of bethylid wasps are larval-pupal ectoparasitoids of *H. hampei*: *Cephalonomia stephanoderis* Bertrem, *C. hyalinipennis* Ashmead, and *Prorops nasuta* Waterson. *Cephalonomia stephanoderis* and *P. nasuta* are of African origin and have been introduced into the Americas where populations have become established. In contrast, *C. hyalinipennis* was first reported as a parasitoid of *H. hampei* in Mexico (Pérez-Lachaud 1998), but was previously documented in Europe, the United States, and Canada (Pérez-Lachaud *et al.* 1999). These three species also fed on *H. hampei* eggs (Jaramillo *et al.* 2006). However, the impact of *C. stephanoderis* and *P. nasuta* has been minimal (Damon 2000; Baker *et al.* 2002). Interspecific competition among these three bethylid species has also been reported in laboratory studies with evidence for aggressive host and brood guarding behavior (Batchelor *et al.* 2006). Furthermore, *C. hyalinipennis* exhibited hyperparasitoid behavior when provided with *C. stephanoderis* and *P. nasuta* larvae, leading Pérez-Lachaud *et al.* (2002, 2004) to conclude that *C. hyalinipennis* is a facultative hyperparasitoid. Because repeated augmentative releases of a single bethylid species yields parasitism levels below 5% (Baker 1999), the potential reduction in efficiency of these parasitoids, if released together, may be negligible. Therefore, a positive economic impact may be derived from the presence of multiple bethylid species, especially if examined on a regional basis.

Phymastichus coffea LaSalle (Hymenoptera: Eulophidae), native to Africa, is a gregarious endoparasitoid of adult female *H. hampei* (Borbón-Martínez 1989) and can parasitize *H. hampei* females within hours of emerging (Jaramillo *et al.* 2006). Once parasitized, female *H. hampei* cease oviposition and usually die after 12 days (Feldhege 1992). Additionally, the highest levels of parasitism were recorded by Jaramillo *et al.* (2005) in berries less than 160 days old, before female *H. hampei* reached the endosperm of the berry, thus preventing damage to the coffee bean. Mass release of *P. coffea* should therefore be timed such that the majority of *H. hampei* have not completely bored into the berries (Jaramillo *et al.* 2005). First introduced in Colombia in 1996, *P. coffea* has become established in North, Central and South America (Baker *et al.* 2002).

Predators.

Heterospilus coffeicola Schmiedeknecht (Hymenoptera: Braconidae) is a wasp native to Africa that preys on *H. hampei*. A female *H. coffeicola* lays one egg per berry and its larva consumes *H. hampei* eggs and larvae (Murphy *et al.* 2001). Due to

difficulties encountered in rearing, *H. coffeicola* has not been used in biological control programs. An unidentified African species of *Leptophloeus* (Coleoptera: Laemophloeidae) has also been observed as a predator of *H. hampei* larvae (Vega *et al.* 1999), and nine ant genera (Hymenoptera: Formicidae) prey on *H. hampei* in Colombia (*Brachymyrmex*, *Crematogaster*, *Paratrechina*, *Pheidole*, *Solenopsis*, and *Wasmannia*; Bustillo *et al.* 2002; Armbrrecht *et al.* 2005) and Mexico (*Azteca*, *Pseudomyrmex* and *Tapinoma*; Infante *et al.* 2003). *Solenopsis picea* Emery is considered to be the most efficient *H. hampei* predator; it enters the berry, removes immature *H. hampei* and carries them back to their nests (Armbrrecht & Gallego 2007). Ultimately the value of generalist predators lies in their role in conservation biological control, thus impacting pest species throughout the season and potentially regulating pest populations when specialist natural enemies are absent.

Recently, a predatory thrips, *Karnyothrips flavipes* (Jones) (Phlaeothripidae), has been observed consuming *H. hampei* eggs and larvae inside coffee berries (Jaramillo 2008). *Karnyothrips flavipes* has a wide distribution, including North, Central and South America, the Pacific region, India, the Mediterranean, Palestine, Egypt, Europe, and South and Central Africa. It is a generalist predator that feeds mainly on scales, mites, whiteflies and other thrips, and is frequently associated with bamboo and other species of Gramineae (Priesner, 1960, 1964). This is the first time *K. flavipes* has been reported as a predator of Coleoptera and associated with non-graminaceous plants.

Entomopathogenic Nematodes and Entomopathogenic Fungi.

In laboratory studies, *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) and *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae), were able to locate and penetrate *H. hampei*-infested berries, resulting in high *H. hampei* mortality (Molina & López 2002). Furthermore, these species are able to reproduce inside immature and adult *H. hampei* (Lara *et al.* 2004). In Mexico, *Metaparasitylenchus hypothenemi* (Tylenchida: Allantonematidae) was observed parasitizing *H. hampei* (Castillo *et al.* 2002). While it does not cause high mortality levels, it significantly impacts female fecundity (Poinar *et al.* 2004).

Beauveria bassiana (Balsamo) Vuillemin (Ascomycota: Hypocreales) is known to infect *H. hampei* adults, and has been reported in many countries (Damon 2000). Application of extremely high doses ($>10^{10}$ spores/tree) is effective in controlling *H. hampei*, but such concentrations are not economically feasible (Posada 1998). Studies aimed at reducing the necessary dosage by increasing the virulence of *B. bassiana* and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) through genetic modifications are in their infancy (Góngora 2005; Pava Ripoll *et al.* 2008). Recent research has attempted to introduce *B. bassiana* as a fungal endophyte in coffee plants, in an attempt to have it become established systemically throughout the plant (Vega *et al.* 2008).

ROLE OF DNA-BASED DETECTION OF PREDATOR-PREY INTERACTIONS.

Tracking trophic interactions by direct observation is not always possible. This is especially true for small, elusive insects such as those that spend nearly all of their life cycles within a host plant. Visual inspection of gut content is only possible when a

portion of the ingested prey is resistant to digestion. By contrast, molecular methods can detect the presence of small amounts of prey in the digestive tracts of predators (Agusti *et al.* 2003; Harper *et al.* 2005; Read *et al.* 2006; Harwood *et al.* 2007; Juen & Traugott 2007; Fournier *et al.* 2008). In these studies, DNA is extracted from the predator and prey species under study and sequenced using universal primers. Mitochondrial genes have been favored because they have a large copy number relative to most nuclear genes and there are less conserved (e.g., COI) and more conserved (e.g., 12S rDNA) areas offering both species- and group-specific primer possibilities. Once sequences are obtained for a group of focal taxa, primer pairs can be designed to exclusively amplify a small region of DNA (usually <300 base pairs) of a prey species. Because the DNA of prey species remains variously intact in the digestive tract of predators for a period of time after predation occurs, prey DNA can be extracted and detected with species-specific primers. Field collected predators can then be screened for predation, enabling an accurate assessment of biological control and examination of trophic interactions that would be extremely difficult to observe directly. These molecular approaches are becoming an essential part of biological control assessment and complement existing antibody-based studies (Hagler & Naranjo 2005) that continue to prove invaluable for examination of large-scale predator-prey interactions in the field. Here, we present preliminary results of the use of *H. hampei*-specific primers to detect *H. hampei* predation by *K. flavipes*.

MATERIALS & METHODS.

Coffee berries were collected from a plantation in western Kenya and dissected to determine the insect fauna of the berries. Additional coffee berries were put into containers from which the emerging insect fauna could be collected. Insects were preserved in >95% ethanol and stored at -20°C. Total DNA was extracted from whole specimens using QIAGEN DNeasy Tissue Kits following the animal tissue protocol. Polymerase chain reaction (PCR) was performed to amplify cytochrome *c* oxidase I (COI) from the insect species that have been found associated with the coffee berry [*Aphanogmus goniozi* Dessart, *H. hampei*, *K. flavipes*, *P. nasuta*, *Tapinoma* sp., Aleyrodidae (Hemiptera) and Drosophilidae (Diptera)] using the primers LCO-1490 and HCO-2198 (Folmer *et al.* 1994). PCR reactions (50µL) consisted of 1X QIAGEN PCR buffer (1.5 mM MgCl₂), 0.2 mM each dNTP, 0.5 mM each primer, 1U QIAGEN *HotStarTaq*® Plus and 5µL template DNA. PCR reactions were carried out in a Bio-Rad thermal cycler. PCR cycling protocols were 94°C for 5 min followed by 50 cycles of 94°C for 45 s, 40°C for 45 s, 72°C for 45 s and a final extension of 72°C for 10 min. Electrophoresis of 10 µL of PCR product in 1.5% SeaKem agarose stained with ethidium bromide was done to determine reaction success. PCR reactions that yielded significant product were purified with QIAGEN MinElute PCR purification kit. Cycle sequencing was carried out in both the forward and reverse directions using the ABI Big-Dye Terminator mix (v. 3.0) in an ABI 9700 thermal cycler, and run out in an ABI 3730xl sequencer.

Forward and reverse COI sequences from the same individual were assembled using AlignIR (v. 2.0). Multiple sequence alignments were done using CLUSTAL_X (Larkin *et al.* 2007). This alignment was used to design two pairs of COI primers (Chapman, Jaramillo, Vega & Harwood, unpublished). One pair was designed to amplify a 646 bp fragment of *K. flavipes* COI to check whether the *K. flavipes* DNA extractions were successful. A second pair was designed to amplify a 185 bp

fragment of *H. hampei* COI, and was screened for cross-reactivity against all other insects that were found associated with the coffee berry including starved *K. flavipes*. This primer pair was used to detect the presence of *H. hampei* DNA in *K. flavipes* DNA extractions. To determine PCR reaction success utilizing the *H. hampei*-specific primers, electrophoresis of 10µL of PCR product in 3% SeaKem agarose was done to separate the 185 bp PCR product from the glycerol-bromophenol blue-based loading dye. Positive controls containing *H. hampei* DNA and negative controls were included in each PCR.

RESULTS.

PCR using the *H. hampei* specific primers was done to examine the feeding relations of *K. flavipes* and confirm specificity of the primers. Figure 2 shows an agarose gel loaded with PCR reactions with DNA from all of the species associated with the coffee berry (listed above). The *H. hampei*-specific primers produced amplicons of expected size from PCRs with extractions of *H. hampei* DNA (Fig. 2: top and bottom: lane 2), *K. flavipes* fed *H. hampei* (Fig. 2: top: lanes 3-7), and *K. flavipes* that emerged from coffee berries (Fig. 2: bottom: lanes 3-5). PCR reactions did not produce products for starved *K. flavipes* (Fig. 2: bottom: lanes 6-8) or any other insect found to be associated with the coffee berry (Fig. 2: top: lanes 8-18).

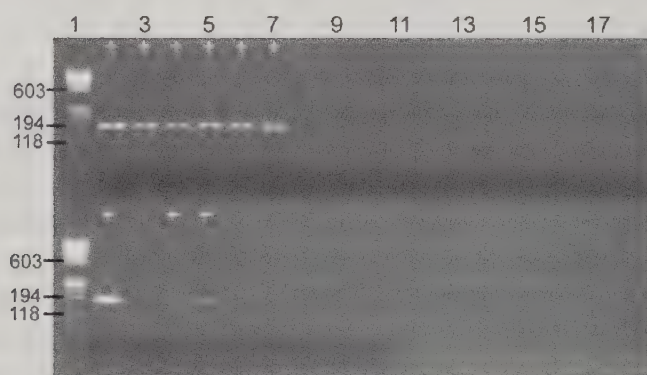


Fig. 2. Agarose gel loaded with PCR products using *H. hampei* specific primers. Numbers across the top indicate lane number, positive results are indicated by “+” and numbers along the side indicate the size (bp) of three bands in the PhiX174/Hae III size standard. Top row: lane 1: size standard; lane 2: *H. hampei* (positive control); lanes 3-7: *K. flavipes* fed with *H. hampei*; lanes 8-9: *Prorops nasuta*; lanes 10-11: *Tapinoma* sp.; lanes 12-13: undetermined Drosophilidae species; lanes 14-15: undetermined Aleyrodidae species; lanes 16-17: *Aphanogmus goniozi*; lane 18: *Heterospilus* sp.; Bottom row: lane 1: size standard; lane 2: *H. hampei* (positive control); lanes 3-5: *K. flavipes* that emerged from coffee berries; lanes 6-8: starved *K. flavipes*; lanes 9-10: negative controls.

DISCUSSION & FUTURE DIRECTIONS.

Our results show that it is possible to detect *H. hampei* DNA from extractions of a tiny (<2mm long) predatory thrips species, *K. flavipes*. We used this technique to demonstrate that *K. flavipes* emerging from coffee berries feed upon *H. hampei*. We are currently in the process of completing a year-long study to decipher the level of

trophic connectedness between this abundant generalist predator and *H. hampei*, an economically damaging pest of coffee. Because *K. flavipes* has a geographic distribution that encompasses *H. hampei*'s range, the results of our continuing study could have important implications for biological control in coffee worldwide. Because the *H. hampei*-specific primers do not amplify the DNA of any of the other insect species associated with these systems, the primers have potential to be used to determine the connectedness of other predators (e.g., ants) to *H. hampei*. Furthermore, because we are compiling DNA sequences from as many of the insects associated with the coffee berry as possible, we have the potential to design species-specific primers for each coffee berry-frequenting insect species. This would enable us to unravel the trophic structure of the coffee berry insect fauna, which could lead to a much improved understanding of the role of natural enemies in control of *H. hampei*.

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DIEL PREDATION PATTERN ASSESSMENT AND EXPLOITATION OF SENTINEL PREY: NEW INTERPRETATIONS OF COMMUNITY & INDIVIDUAL BEHAVIORS

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ABSTRACT.

Little effort has been made to characterize the diel pattern of predation on insect pests in the field, particularly predatory events that occur nocturnally. Round-the-clock observations in systems such as potatoes, cotton, soybean, maize, and ley crops under varying cultural practices, and woodlands, with additional published studies, highlight several important considerations when working on predator communities:

- Predator communities differ greatly between day and night. Depending on the system, cursorial spiders, carabid and other predatory beetles, cockroaches, ants, and earwigs, are important after dark.
- Predators have distinct diel patterns of behavior, which do not conform simply to a nocturnal/diurnal dichotomy; circular statistics are important in diel analysis.
- Diurnal appraisals are at best biased; predators causing significant mortality to target pests may be completely overlooked.
- Sentinel (emplaced) prey are uniquely useful, provided they represent target pests and are stationed realistically. Predator taxa and diversity vary greatly with prey type, habitat, and position.
- Combining direct observations with other methods (including DNA and protein-based molecular tools) strengthens predation assessments
- Disturbed habitats tend to host lower predator diversity and only during limited diel windows; cultural techniques which enhance complexity in agricultural habitats may expand the diel period for higher intensity and diversity of predation on pests.

We conclude that nocturnal predation must be addressed explicitly to accurately characterize predator-prey systems, and that round-the-clock observation of sentinel prey is a key technique for assessing pest suppression as an ecosystem service

INTRODUCTION.

Predation in agricultural systems is at least as common after dark, as during the day. Yet research which investigates predation throughout the diel cycle is rare indeed. Over 30 years later we can still echo the sentiment of Vickerman & Sunderland (1975) that “the nocturnal activity of terrestrial invertebrates seems to have been largely overlooked in crop ecosystems.

Both natural and managed ecosystems show diel rhythms in predator activity, including predation events. The diel patterns vary by species, season, and habitat; furthermore, community patterns vary over these same temporal and ecological dimensions. To identify and characterize important ecological processes including predation, and to build effective management methods for sustainable agricultural systems, it is essential to have full knowledge of the multiple dimensions of diel activity.

It is important not just to think or design studies in terms of “nocturnal” versus “diurnal”, nor to classify species or species groups into such categories. Conditions and behavior vary over the entire 24 hours; for instance, temperature and dew are very different from beginning to end of scotophase, with great influence on predator behavior.

Early ecological workers considering mainly the Carabidae (e.g. Williams 1959; Thiele & Weber 1968) showed a variety of patterns of activity, as measured typically by pitfall capture or actographs of recently-caught beetles in captivity. Dennison & Hodkinson (1983) related woodland carabid and staphylinid diel activity patterns to species, body size, and season, with a variety of nocturnal, diurnal, crepuscular, and intermediate or combined activity patterns. Dennison & Hodkinson (1984) used presence-absence diel activity data for 13 carabids, 9 staphylinids and one leiodid, to cluster the species in a “diurnal activity dendrogram” according to average linkage clustering. This resulted in 3 loose clusters as well as two unique species. The pattern was far from a simple behavioral dichotomy of nocturnal versus diurnal. In agricultural systems, Luff (1978) and Kiritani *et al.* (1972) observed similarly diverse diel patterns, respectively, in carabids of an English vegetable system, and in spider predators of the green rice leafhopper *Nephotettix cincticeps* Uhler (Homoptera: Cicadellidae), in Japanese rice paddies.

That the diel pattern of occurrence, activity or predation might vary according to habitat was proposed by Park (1941), who viewed diel patterns from the perspective of niche-filling, with more symmetric diel activity accompanying advanced community succession. However, only a very few studies have taken place in both natural and agricultural systems to examine these trends in detail, especially in terms of predation risk to herbivores.

Activity of predators is important, but with biological control, and particularly in agroecosystems, the focus is on the prey, usually pest species, of concern to crop protection. Making the prey the focus of interest makes predation events themselves, rather than predator activity (movement) or location within the ecosystem, and allows the best understanding of ecosystem services in terms of desired, practical biological control.

The focus on particular prey, in particular pests in agricultural systems, is likely to yield more useful results about biological control, especially predation risk, than is general sampling or observation. Molecular methods may focus on prey-specific molecules with valuable results, but the ecological context of these markers must be established in order to confirm how (predation, scavenging, secondary predation), where (within the crop system) and when (during the diel cycle) the prey is obtained by different predators. This will allow proper application of molecular tools in determining the importance of the predator in suppressing the pest of interest.

Use of sentinel prey, either the pest of interest or a factitious (surrogate) species which has been demonstrated to be equivalent as a prey item, is extremely valuable in assessing the intensity of predation and the species responsible for biological control. It may be especially useful where pest density is insufficient to observe adequate numbers (sometimes as a result of very effective biological control!). Kidd & Jervis (1996) describe the caveats in deploying sentinel prey, including positioning, quality, and density considerations.

Here, we describe the deployment of eggmasses of Colorado potato beetle (*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)) in potato crops, monitored day and night to determine predator occurrence and predation on prey of interest. In addition, we discuss additional studies undertaken by the authors, using sentinel eggs of Lepidoptera in field crops such as cotton, soybeans, and corn, and sentinel larvae in vegetable and field crops under organic transition.

MATERIALS AND METHODS.

The field site was a 1-ha potato field in Beltsville, Maryland, USA. Once in July 2006, and at 3 times during 2007, during continuous 48-hour periods we observed 240 field-collected sentinel egg masses of Colorado potato beetle for damage and presence of arthropods on or nearby, every 3 h, at 0200, 0500, 0800, 1100, 1400, 1700, 2000, and 2300 hours.

Eggs were numbered and photographed prior to attachment to leaves in the field using staples. Previous work had shown that attachment to the tops of leaves resulted in similar predation to attachment underneath, in spite of the fact that the large majority of eggs are laid by females underneath leaves, perhaps to prevent desiccation. Plots of 20 plants each were placed in 3 different cultural treatments of potatoes (conventional tilled, killed rye cover crop, & killed vetch cover crop), with 2 replicates in each of two fields, planted in April with cultivar 'Kennebec' and minimal pesticide treatment. The results shown are for the sample period 26-28 July 2006, and do not show cultural effects.

Observations were made with a minimum of disturbance, using red LED headlamps when needed after dark. All egg masses with $\geq 50\%$ estimated damage were replaced immediately. Damaged egg masses were enumerated to quantify depletion (before minus after).

To analyze the diel patterns, we used circular statistics (Batschelet 1981; Fisher 1993; Zar JH. 1999) with Oriana software (Kovach 2004). Circular statistics and polar plots are essential to analysis and visualization of cyclical temporal data.

RESULTS.

Of the 255 predation events observed in July 2006, 56% occurred after dark, with the fewest observed during the afternoon hours. The natural enemy complex is dominated by beetles and true bugs (Table 1). The most frequently observed predator in 2006, larval *Chauliognathus* sp. (Coleoptera: Cantharidae), has not been reported previously as a CPB egg predator (Ferro 1994; Heimpel & Hough-Goldstein 1992; Hilbeck *et al.* 1997).

The diel pattern of occurrence of predators on sentinel eggmasses is unique by species, with the exception that Pentatomidae and *Geocoris* sp. (Hemiptera: Geocoridae) have very similar patterns of occurrence (Table 1). *Chauliognathus* sp. has a late-nocturnal peak of abundance, corresponding to the period of heavy dew on plants (Fig. 1). In contrast, *Lebia grandis* Hentz adults (Coleoptera: Carabidae) are primarily early night-active, ceasing activity after midnight but becoming active again typically soon after dawn. *Lebia grandis* egg consumption (not shown) exceeded that of all other predators. Bugs were almost exclusively diurnally predatory on CPB eggs. Among pentatomids, *Perillus bioculatus* (F.) is more specialized on CPB than is *Podisus maculiventris* (Say), and was the dominant species, as nymphs, in our study. Big-eyed bugs (*Geocoris* sp.) was strongly diurnal based on predation events; adults and nymphs were equally represented on CPB eggmasses. However, their primary prey was probably potato leafhopper (*Empoasca fabae* (Harris) (Hemiptera: Cicadellidae) nymphs, which were abundant. *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) is an extreme generalist feeder abundant in some potato fields, particularly following a corn rotation crop. Adults are diurnal and crepuscular feeders, with activity most broadly distributed of all predators.

Figure 2 shows the abundance and species composition of predators on eggmasses by sample period. Predation was least prevalent in the mid-afternoon, and most prevalent late in the scotophase. Sampling during one period of the 24-hour diel cycle could yield very different conclusions: communities were as little as 10% similar between 5:00 and 17:00 (percent similarity, Renkonen 1938, sum of the minimum of proportion by species). In particular, the predator responsible for the most egg consumption, *L. grandis*, was completely absent on egg masses at mid-day during this sample period, in spite of being abundant at night (Fig. 2). Certain periods showed rapid temporal community change in the three hours between samplings: from 5:00 to 8:00 and from 8:00 to 11:00 there are 42.0% and 48.0% respective changes in community composition (1 minus the percent similarity), and again from 17:00 to 20:00 and from 20:00 to 23:00 there are changes of 27.5% and 49.5% respectively. Other successive time periods were very similar, in particular from 23:00 to 2:00 the difference was only 7.1%.

Table 1. Diel pattern of occurrence all predators on Colorado potato beetle egg masses on potato foliage, 26-28 July 2006, Beltsville, Maryland, USA. Mean time vector in 24-hour time, with p-value, according to Rayleigh’s test, the null hypothesis of which is uniformity of occurrence by time of day.

Species	occurrences	mean time vector	p-value	Watson’s F-test for separation
<i>Chauliognathus</i> sp.	121	3:22	<0.005	a
<i>Lebia grandis</i>	18	0:17	<0.005	b
<i>Coleomegilla maculata</i>	43	14:20	0.24	abc
<i>Geocoris</i> sp.	36	13:40	<0.005	c
Pentatomidae	24	13:04	0.02	c

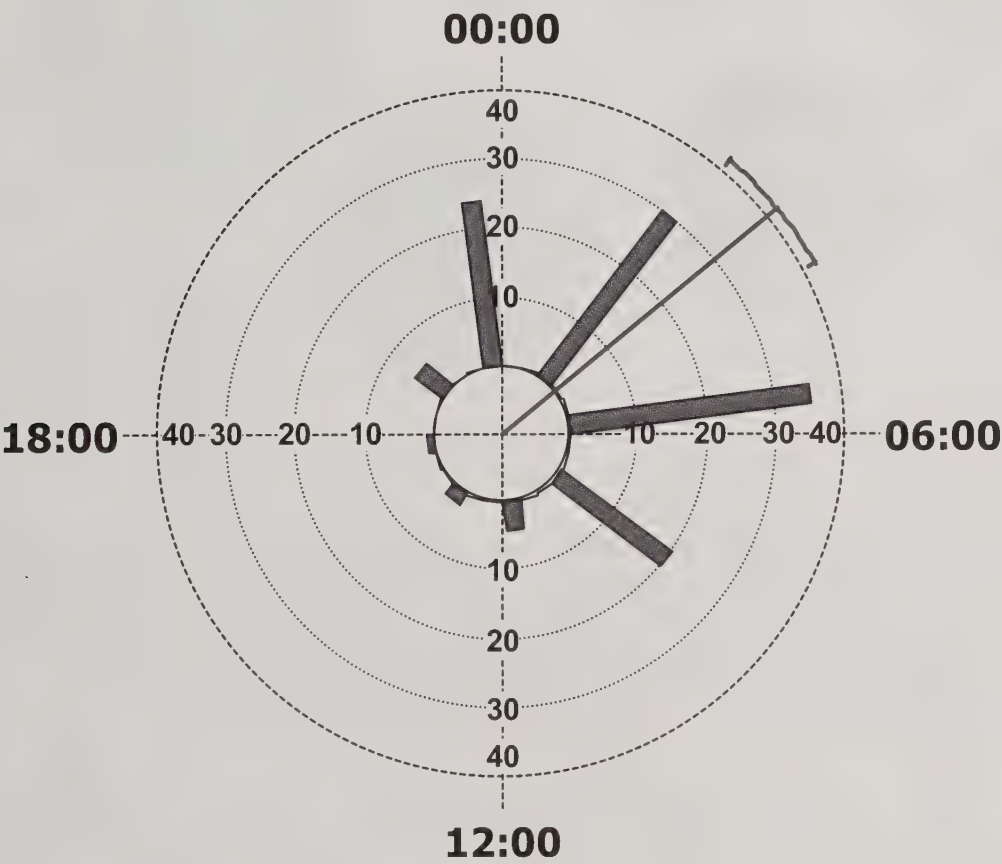


Fig. 1. Diel pattern of occurrence of cantharid larvae, *Chauliognathus* sp., on Colorado potato beetle egg masses on potato foliage, 26-28 July 2006, Beltsville, Maryland, USA. Mean time vectors and 95% confidence interval shown with 00:00 as midnight.

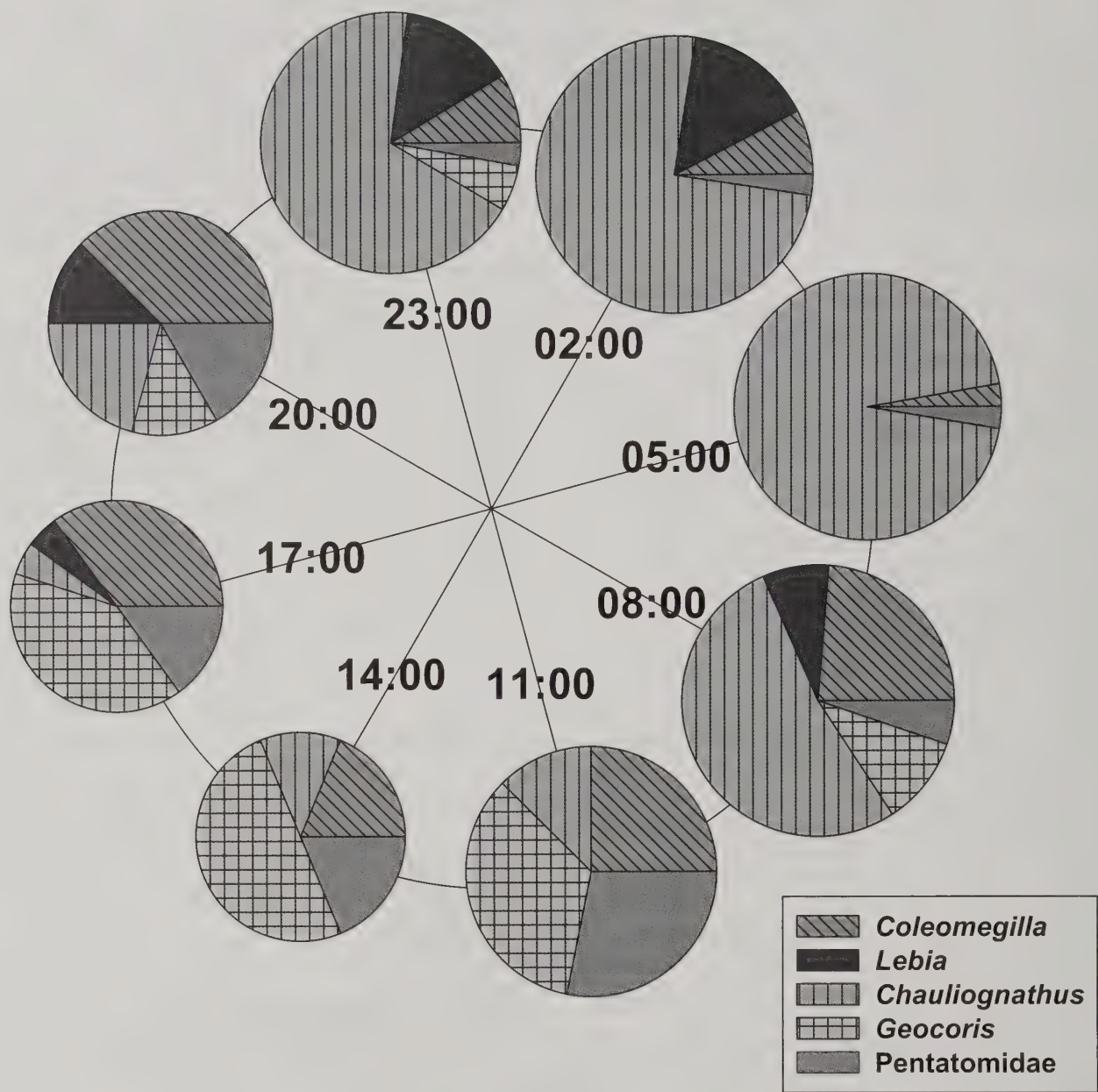


Fig. 2. Occurrence of predators on Colorado potato beetle egg masses on potato foliage, 26-28 July 2006, Beltsville, Maryland, USA. Areas of pies are proportional to number of occurrences during the sample period (every 3 hours for 48 hours), with a maximum of 40 occurrences at 2:00 and a minimum of 16 at 14:00.

DISCUSSION.

Our results in potato are consistent with those of Pfannenstiel (2005) and Pfannenstiel & Yeargan (2002) in field crops, in that nocturnal predation was equal to, or exceeded, diurnal predation. However, compared to the dominance of cursorial spiders and ants feeding on sentinel lepidoptera eggs at night in Texas cotton, corn, and soybeans, our plant canopies hosted few of these predators and they were very rarely found on eggmasses. Colorado potato beetle eggs as well as larvae represent a distinct prey item from lepidoptera eggs, which may not attract

spider or ant predation. The predator complex of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) for Kentucky soybean and sweet corn is more similar to our observed fauna in Maryland, with the exception that in Maryland, hemipteran predator species were different, and phalangids were never important. In 2006 sampling, we found little difference between cultural treatments (data not shown), perhaps because canopy closure and relatively small plot size resulted in a relatively uniform distribution of predators among plots.

In contrast, Lundgren *et al.* (2006) aimed to create strong cultural differences among lower-input pasture system and higher-input tilled vegetable and cash grain systems. The low-input system, transitioning to organic practices, had little diel variation in soil-surface predation rates of tethered factitious prey caterpillars (greater waxmoth, *Galleria mellonella* L. [Lepidoptera: Pyralidae]). In contrast, an intensively-managed vegetable system showed significantly higher predation during a 3.5-hour night-time sampling period, than either morning or afternoon 3.5-hour observation windows. Predation was not related to pitfall trap captures of potential predators. However, predation was very significantly correlated with overall abundance of insectivorous predators, as measured by quadrat sampling in the different systems. This correlation was in spite of the fact that observed predation involved only a fraction of the predators sampled in the quadrats, and none of the most abundant group, spiders.

Frank & Shrewsbury (2004) tested the effect of nearby conservation strips for predation on tethered black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) larvae and pupae, and showed a significant effect on larval predation at 6m and 10m distance but not at two closer distances nor at any distance on pupal predation. Again in this case the predation risk showed no relationship to pitfall trap catches, reinforcing the importance of prey-centered measurement of predation, rather than interception of moving epigeal predators.

Clearly the reluctance of entomologists to undertake time-consuming night-time observations has limited the valuable data otherwise available. One technological fix for this is the technique of unattended video recording of sentinel prey carried out in Germany by Meyhöfer (2001). Using 16 monochromatic surveillance cameras illuminated at night by infrared LEDs, he observed colonies of parasitized and unparasitized aphids, *Aphis fabae* Scopoli (Homoptera: Aphididae) in German sugar beets. These continuous 24-hour video observations allowed detailed study of parasitoid survival with predator presence and time spent in prey patches, and to identify individual predators eating parasitized aphids. The video recordings also allowed detailed determination of diel patterns of predation by species groups.

The video recording technique has also been employed in New Zealand by Merfield *et al.* (2004) and Frank *et al.* (2007). Time-lapse video recorders were allowed to run for 12 hours during the scotophase only. Merfield *et al.* (2004) used blowfly, *Calliphora stygia* F. (Diptera: Calliphoridae), live eggs as factitious prey and thereby demonstrated differences in predation patterns based on field borders as well as a previously undiscovered commensal interaction between predatory mites and the harvestman *Phalangium opilio* L. Frank *et al.* (2007) demonstrated differences in predator complex, but not overall predation pressure, between vineyard floor and canopy, where the prey were tethered larval light brown apple moths, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae).

Other technologies can be coupled with diel pattern studies to yield insights into predator dynamics. Vickerman & Sunderland (1975), working in English small grains, examined nocturnal activity, demonstrating changing distribution along a vertical axis using manual sweep-netting (for the plant canopy) and D-vac sampling (principally for the soil surface). They also examined predator guts for recent aphid predation, using physical examination and antigen testing. Polyphagous predators, including staphylinids, carabids, earwigs, and spiders, were found at night, particularly in the plant canopy, and these were more likely to have fed recently on aphids at night than during the day. More recently, Lundgren *et al.* (in press) used a timed pitfall trap in concert with augmentation of western corn rootworm (*Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae) and rootworm-specific quantitative PCR to elucidate diel and seasonal patterns as well as to discover several new predators of this key pest in South Dakota corn.

Remedying the serious nocturnal deficiency in agroecology will take more than video cameras and rotating pitfall traps! We ignore the scotophase at our peril. As the observations in Maryland potatoes show, novel predators may be present and undetected unless nocturnal observations are undertaken. Similarly, the discovery of an Asian cockroach, *Blattella asahinai* (Dictyoptera: Blattellidae) as an important predator in soybean and possibly other crops in southern USA (Pfannenstiel *et al.* 2008) was only by virtue of ongoing nocturnal ecological research. This exotic species had probably been present for over 20 years in the USA (Pfannenstiel *et al.* 2008).

More generally, we as ecologists need to take the entire diel cycle into account, when contemplating “ecological engineering for pest management” (Gurr *et al.* 2004). Nectaries, for instance, are not just to the benefit of syrphids and parasitic hymenoptera; in the scotophase spiders which can be important agricultural predators (Pfannenstiel 2008) make use of floral and extrafloral nectaries (Taylor & Pfannenstiel 2008). On the potential negative side, pestiferous noctuid moths and other herbivores augment their fecundity by nocturnal nectarivory and pollinivory (Wäckers *et al.* 2007).

Limited information available (e.g. Lundgren *et al.* 2006, Lundgren unpublished) suggests that mulching, reduced tillage, more closed and diversified canopies all can serve to expand the diel range of predation pressure in agricultural systems over a broader time period, typically from night into the day. These practices serve both to buffer climatic (temperature, humidity, light and wind) extremes, and to mimic more generally the later successional stages of natural ecosystems, such that a perennialization effect takes place, even in agroecosystems consisting largely of annual cash and cover crops.

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THE USE OF ELISA TECHNIQUES TO CLARIFY PREDATOR/PREY INTERACTIONS IN COTTON

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ABSTRACT.

The basis to understanding food webs is knowing which predators feed on which prey. This is particularly difficult to do with arthropods. To help solve this problem, sandwich enzyme-linked immunosorbent assays (ELISAs) have been developed in which prey are marked with an exotic vertebrate protein, and potential predators tested for that protein. However, it is unclear how effective the technique is with different prey species and different predators. This paper compares the effectiveness of marking two important emerging pests in Australian cotton: the cotton aphid and the green mirid, with marking of an established pest, cotton bollworm eggs; and the ability of predators to take up the mark from these prey.

The results suggest that although the eggs were the easiest prey to mark and had the longest retention of the mark, predators feeding on mirid prey were marked more strongly. Predators differed in their unmarked base absorbance levels, but this difference was slight compared to differences between different phylogenetic groups and their likelihood to assimilate the protein mark. For example, the three spiders tested were very likely to become marked, while the damsel bug was not. Differences in feeding techniques may contribute to this variability.

Sandwich ELISAs testing for the presence of exotic proteins are a very sensitive and effective way of identifying potential predators of marked prey, but caution must be taken when comparing predators from different phylogenetic groups using different foraging methods. In addition, some predators became marked without feeding on the prey, indicating that care must be taken regarding the release of marked animals into the field to minimise incidental marking of predators. Nevertheless, the technique has the potential to reveal cryptic predator/ prey interactions and therefore is a useful tool in the management of pests using conservation biological control.

INTRODUCTION.

The widespread use of transgenic Bt cotton to control damage from the cotton boll worm (*Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)) has seen a dramatic drop in the amount of insecticide applied to crops, which has enhanced Integrated Pest Management (IPM) in cotton (Wilson *et al.* 2004). However, with the demise of *H. armigera* there has been an increase in sucking pests such as aphids and mirids. The management of these emerging pests with broad-spectrum insecticides threatens to disrupt IPM. One way to maintain the recent decline of insecticide use in cotton is to identify the predators which attack sucking pests so that

such predators can be incorporated into conservation biological control. However, our knowledge of the predators of these pests is poor, especially for the green mirid *Creontiades dilutus* (Stål) (Hemiptera: Miridae). Identifying the main predators of hemipteran pests would enhance their control within the existing IPM framework.

Unfortunately, identifying predator/prey linkages within invertebrate communities is problematic, as these animals are often cryptic or nocturnal (Hagler & Cohen 1990) and leave little evidence of a predation event. The development of prey-specific immunological and DNA-based techniques to detect prey in the gut contents of individual predators has been a major advance to understanding predator/prey interactions (Greenstone 1996; Sheppard & Harwood 2005).

One of these techniques showing particular promise is the sandwich enzyme-linked immunosorbent assay (sandwich ELISA) used to detect an exotic protein marker that is applied to the target prey (Hagler & Durand 1994; Hagler 2006). This technique has often been used to mark insects for mark/recapture studies (Hagler & Naranjo 2004; Blackmer *et al.* 2006). Mansfield *et al.* (2008) further developed this technique for predator prey interactions in Australian cotton by marking *Helicoverpa armigera* eggs with rabbit IgG protein, and then using the sandwich ELISA to detect its presence in two key predatory beetles: *Dicranolaius bellulus* (Guerin-Meneville) (Coleoptera: Melyridae) and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). They showed that the technique was more specific and sensitive than an anti-*H. armigera* indirect ELISA.

The aim of this paper is to develop the anti-rabbit IgG sandwich ELISA further by testing it on two emerging pests: the cotton aphid (*Aphis gossypii* Glover (Hemiptera: Aphididae), hereafter referred to as “aphids”) and the green mirid (*C. dilutus*, hereafter referred to as “mirids”) and comparing the results to those for *H. armigera* eggs (hereafter referred to as “eggs”). In particular, we will establish if mirids and aphids can be marked, how long the mark lasts, the uptake of the mark by predators and whether this technique could be adapted to field trials involving a range of predators.

METHODS.

Testing marked prey.

The initial step in this work was to see if marking mirids and aphids with rabbit IgG protein was comparable to marking eggs in terms of the ease of marking, and its strength and permanence. Alive eggs, mirids and aphids were marked by dunking, spraying, or sucking. The concentration of the rabbit protein in all cases was 5 mg/ml.

Dunking - The animal was placed in a small plastic specimen vial (diameter 50 mm, height 60 mm) containing a small amount of rabbit protein. After it had contacted the protein it was removed from the vial and allowed to dry. This technique was most successful with eggs. Mirids and aphids were anaesthetised with CO₂ before being dunked.

Spraying - The animals were sprayed with a Nebulizer which produces a very fine, fog-like mist (Hagler 1997). Aphids were sprayed for 2 min while standing on a

leaf. Mirids were placed in a specimen vial, anaesthetised with CO₂, and then sprayed for 1 min, shaken slightly, and sprayed again for 1 min (making a total of 2 min). Eggs were sprayed for 2 min in a specimen vial.

Sucking – This method was only used with aphids. Aphids were placed in film canisters (diameter 20 mm, height 50 mm) which were sealed on top with Parafilm®. A large drop of rabbit protein in a 10% sugar solution was placed on top of the Parafilm®, and was covered with another layer of Parafilm®. The aphids were left in the container for 24 hours. Only aphids found on the Parafilm® lid (and therefore may have been feeding) were used in this test.

Marked prey items were tested, or stored at -80°C in readiness to be tested, 0, 6, 12, 24, 48 or 96 hours after marking. Eggs killed by freezing and then thawed were also tested 1 and 2 weeks after marking. Between marking and testing, eggs were left in Eppendorf tubes at room temperature; aphids were placed in specimen vials on cotton leaves at room temperature; and mirids were placed in specimen vials containing a bean and some unmarked eggs (their normal diet). Mirids were only tested 0, 24, 48, and 96 hours after marking. Some marked eggs were allowed to hatch. These were tested 0, 6, 24, 48, and 96 hours after hatching. The significance level for the absorbance readings was determined by taking the negative controls (8 unmarked prey/ plate) and calculating the mean + (3*SD). Absorbance readings of marked prey above this level indicated that the prey had retained the mark.

Testing predators with marked prey.

Predators were placed in a small clean Petri dish with either marked or unmarked prey and left for 30 min., after which they were checked to see if they had eaten (as indicated by the eaten remains) or were still eating the prey. The predators were then removed from the Petri dish and placed in a clean vial. Predators were tested using ELISA techniques to see if they were marked 0, 6, 24, 48, and 72 h after being exposed to the prey. Predators which fed on unmarked prey were used as the negative control.

Predators are referred to by their generic name except for the jumping spiders, which were not identified to species and are collectively referred to by family (Salticidae). The *Oxyopes* (Araneae: Oxyopidae) (lynx spiders) used in this work were one of three species found in cotton in the Namoi Valley (NSW Australia): *O. molarius* L. Koch, *O. amoenus* L. Koch, and *O. gracilipes* (White) (Whitehouse & Grimshaw 2007) of which *O. molarius* is the most common. The other predators used in this study were: yellow night stalkers: *Cheiracanthium* spp. (Araneae: Clubionidae); red & blue beetles: *D. bellulus*; white-collared ladybird: *H. variegata*; brown smudge bug: *Deraeocoris signatus* (Distant) (Hemiptera: Miridae); damsel bug: *Nabis kinbergii* Reuter (Hemiptera: Nabidae); and the big eyed bug *Geocoris lubra* Kirkaldy (Hemiptera: Geocoridae). Specimens of these animals have been deposited within the collection of the Australian Cotton Research Institute at Narrabri.

Anti-rabbit IgG sandwich ELISA Protocol.

To test if pests or predators had been marked by the rabbit protein, each animal to be tested was placed individually in an Eppendorf tube and stored in a -80 °C

freezer until needed. The sample was then ground in 500 µl of Tris-buffered saline (TBS, pH 7.5) in the Eppendorf tube, centrifuged for 3 min at 1400 rpm, and then stored in the -80 °C freezer overnight. The sandwich ELISA is described in detail by Hagler (2006). Each assay plate was coated with 100 µl per well of anti-rabbit IgG (product no. R2004, Sigma-Aldrich, Castle Hill, NSW 1765) diluted 1:500 in de-ionized ultra pure water and incubated overnight at 4°C. The next day the primary antibody was discarded and a 1% solution of non-fat dry milk in distilled water was added to each well for 30 min at 27°C to block unoccupied antigenic sites. The milk solution was then discarded and a 100 µl aliquot of each crushed arthropod sample was added to each well and incubated for 1 h at 27°C. The sample was discarded and the plate washed three times with TBS-Tween 20 (0.05%) and twice with TBS. Anti-rabbit IgG peroxidase conjugate (product no. A6154, Sigma-Aldrich) diluted 1:1000 in 1% milk was added to each well (100 µl) and incubated for 1h at 27°C. This solution was then discarded and the plate washed as before. Finally, 100 µl of HRP substrate solution was added to each well and incubated for 2h at 27°C. The absorbance of each well was read at 415 nm. Each plate included negative controls, positive controls and reagent blanks.

RESULTS.

Testing marked prey

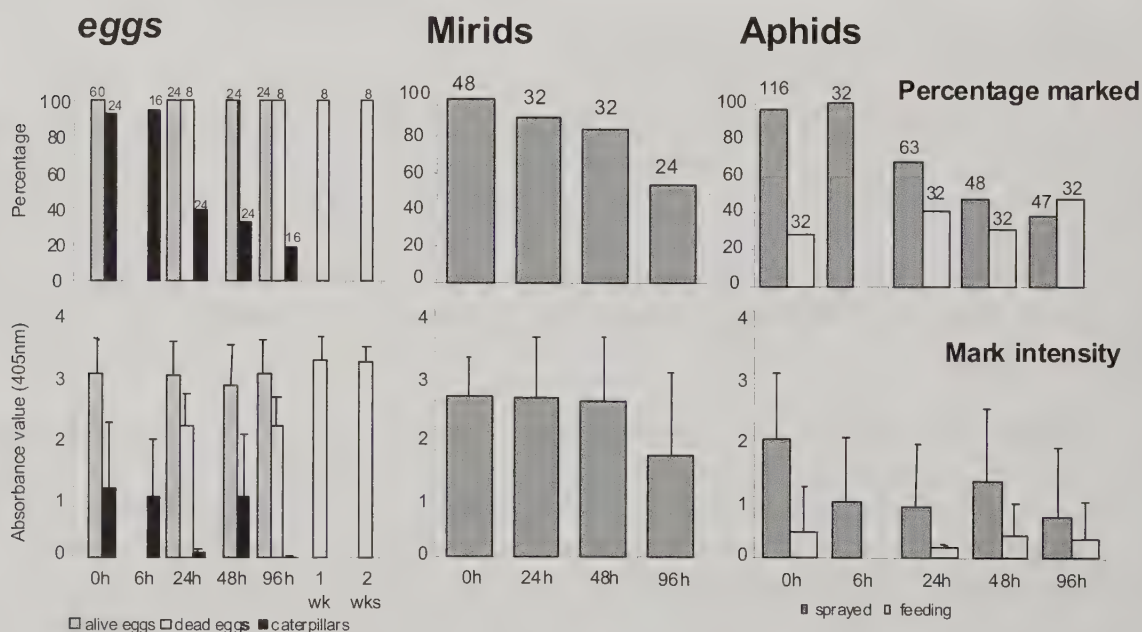


Fig. 1. Percentage of eggs, mirids and aphids that remained marked, and the intensity of the mark, after various time intervals. All animals were marked with rabbit protein by spraying, except “feeding” aphids, which were marked by sucking. The number above the percentage columns are the total number of animals tested. The time interval refers to the time since marking for the eggs, mirids and aphids (and the time since hatching for the caterpillars). The mark intensity was calculated using only samples that recorded a positive mark.

All eggs were easily marked and stayed marked for up to 2 weeks, with high absorbance values (Fig. 1) indicating the presence of the rabbit IgG protein marker. Even caterpillars hatching from their marked eggs retained a mark for 24 h (although not at a high intensity) before the proportion of marked animals began to fall. Mirids were easily marked, but the proportion marked and the mark intensity declined after a few days. Aphids were difficult to mark. External marking was the most effective for aphids, but the proportion of animals marked and the mark intensity dropped off after 6 h. This loss of marking intensity is possibly due to the aphid moulting and their rapid life cycle, combined with their waxy cuticle. We tried to counteract the effect of the waxy cuticle by adding Maxx Organosilicone Surfactant to the protein solution, but as this had no effect on the results, we stopped using it (unpublished data). The percentage of aphids marked by feeding was very low (30%) and the mark intensity was also low.

Testing Predators.

The 151 negative controls in this experiment, which were spread among the ELISA plates, were predators that ate unmarked prey. Of these, three species groups were represented by only one or two samples (*Iridomyrmex* =2, *Deraeocoris* =1, *Geocoris* =1). The rest were represented by more than 20 samples, although not all of these six predators ate all three prey types as some predators did not eat some prey.

The six predators with more than 20 samples were then tested for outliers using box-whisker plots in Genstat 11.1. This revealed that the distribution of absorbencies for the six species was not normal, with 9 “far” outliers (more than 3 times the interquartile range beyond the quartiles) spread among the predators. These extreme data points were excluded from further calculations. A REML analysis revealed that prey type had no effect on the absorbance readings ($F_{1, 122} = 0$; $P = 0.997$) while the predator had a significant effect ($F_{5, 121} = 3.25$; $P = 0.009$). There was also a significant difference between Orders (predicted means of Araneae, Coleoptera, and Hemiptera respectively = 0.034, 0.064, 0.023; $F_{2, 133} = 5.1$; $P = 0.007$) but not within Orders (Araneae: $F_{2, 43} = 2.1$; $P = 0.139$; Coleoptera: $F_{1, 48} = 0.3$; $P = 0.59$; Hemiptera were only represented by one species). Critical absorbance levels (mean+3*SD) were calculated for the three Orders across all plates, and for all predators combined (Araneae = 0.173, Hemiptera = 0.174, Coleoptera = 0.279, All = 0.222). The “All” critical absorbance was used with marked predators which did not belong to either Hemiptera, Coleoptera or Araneae.

Marking predators: We tested 672 predators, belonging to seven taxa, for their likelihood to become marked after eating aphids, mirids or eggs. We found that the spiders (Oxyopes, Cheiracanthium and Salticidae) in particular were well marked, with over 70% of all species obtaining a mark from eating marked prey (Fig. 2). About 30% of the beetles which fed on marked prey obtained a mark, while 25% of *N. kinbergii* became marked. Many predators which didn't eat the prey also became marked, presumably by encountering the marked prey. In particular, over 20% of spiders were marked even though they had not fed on the prey (Fig. 2).

Mark intensity: The absorbance readings differed between predators but unlike the control predators (fed unmarked prey), the prey consumed influenced the

absorbance readings (Fig. 3; REML; effect of predator: $F_{5, 138} = 9.79$; $P < 0.001$; effect of prey type: $F_{2, 139} = 6.48$; $P = 0.002$; no interaction effect). Again the most strongly marked predators were spiders, with beetles and bugs showing similar absorbance readings. Predators which had eaten mirids were also more strongly marked, than those that had eaten eggs or aphids.

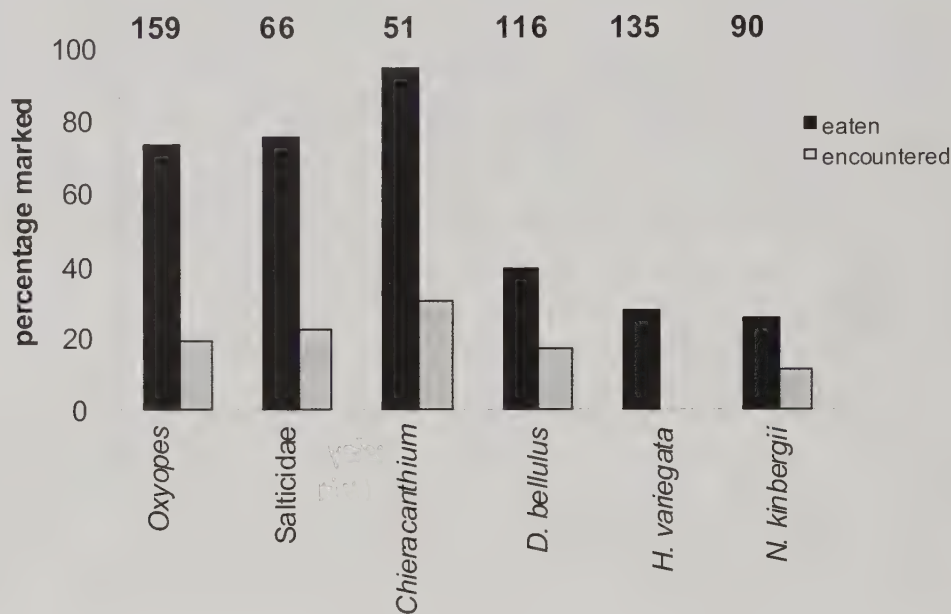


Fig. 2. Percentage of predators marked after feeding on, or just encountering marked prey (all prey species combined). The number above the columns indicates total number of predators tested.

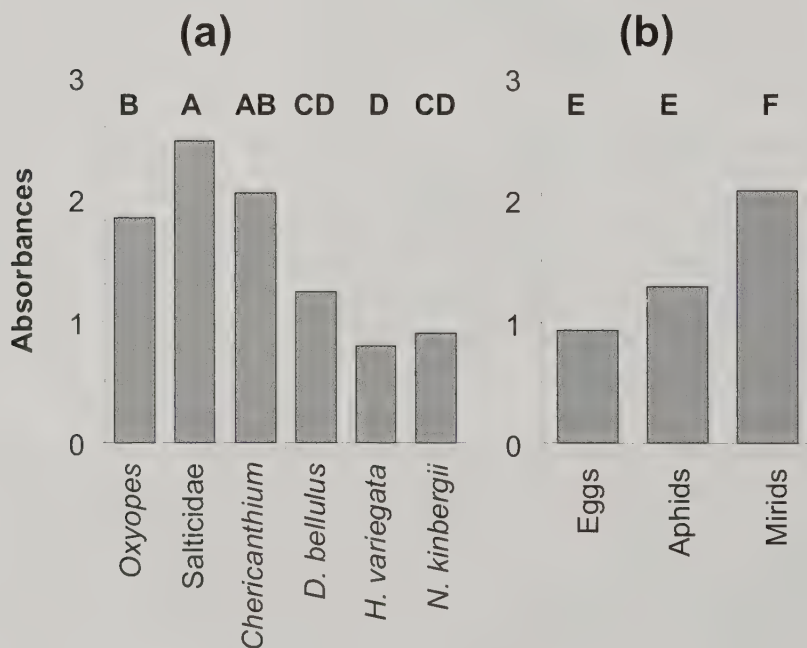


Fig. 3. Estimated absorbance means (using REML) for predators marked by feeding on marked prey: (a) are the absorbance means of the predators, (b) shows the absorbance means of the predators which attacked specific prey. The same letters above a column indicate no significant difference between those columns.

Predators that had not fed but were marked positive presumably from encountering marked prey did not show different absorbance levels in respect to either predator type (ANOVA, $F_{4, 40} = 1.64$; $P = 0.182$) or prey encountered (ANOVA, $F_{2, 42} = 0.27$; $P = 0.767$).

Prey attack rates: We compared the number of prey eaten by the different predators to see if, under laboratory conditions, they varied in their likelihood to attack different prey. We excluded *Iridomyrmex* ants from the analysis because they were behaving aberrantly and *D. signatus* because the sample size was too small. We found that predators varied in their likelihood to attack different prey ($\chi^2 = 300$, $df = 10$, $P < 0.001$; Fig. 4). The spiders (*Oxyopes*, Salticidae and *Cheiracanthium*) were significantly less likely to attack eggs than the other predators, and *Oxyopes* and Salticidae were more likely to attack mirids. *H. variegata* were significantly more likely to attack aphids, and less likely to attack mirids. The beetle *D. bellulus* was more likely to attack eggs, and less likely to attack mirids (Fig. 4).

We then tested to see if there was a significant difference between the percentage of predators which ate prey, and the percentage marked positive for feeding on prey. We found that there was an overall difference between the two groups ($\chi^2 = 201$, $df = 17$, $P < 0.001$; Fig. 4). In particular, *Oxyopes* were over-marked for eggs, *Cheiracanthium* were over-marked for eggs and mirids, *H. variegata* were under-marked for aphids, and *N. kinbergii* were undermarked for eggs and aphids.

DISCUSSION.

Marked prey.

The results presented here show that mirids, and to a lesser extent aphids, can be effectively marked with rabbit protein and used to identify the predators of these animals. The work also showed that it was possible to mark eggs and then follow the activities of the neonates that emerged. (Presumably the neonates obtained the mark from eating their egg shells). Marked mirids in particular were effective to the extent that predators which consumed marked mirids had absorbance readings higher than those that consumed marked eggs. The rabbit protein marker on mirids was stable for 24 h, which would allow sufficient time for marked animals to be released in the field and consumed before sampling for predators needed to begin. The markings on aphids, however, deteriorated quickly, probably due to the rate at which these insects moult. Surveys for predators of aphids would need to be conducted about 6 hours after the marked aphids were released.

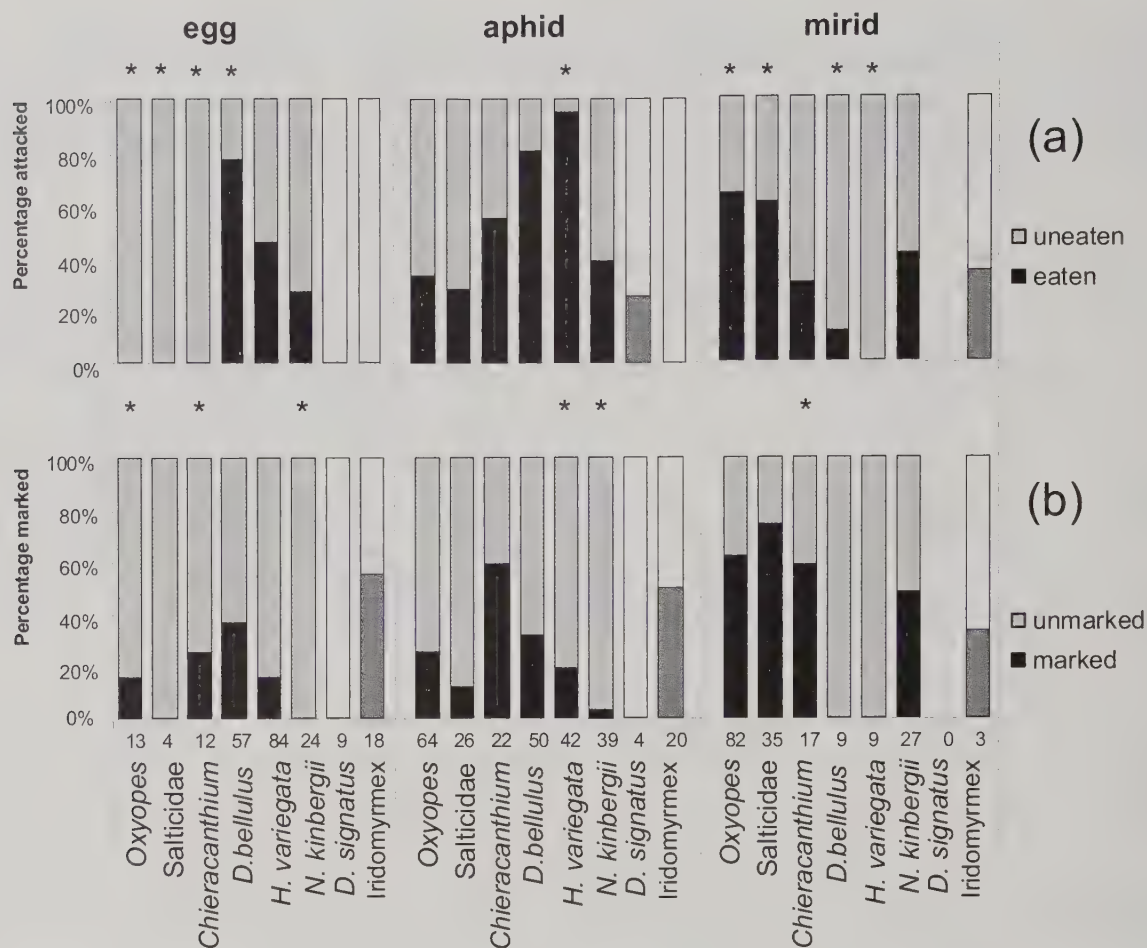


Fig. 4. The percentage of predators that attacked marked prey (a) and the number which were marked positive for feeding on marked prey (b). The * above the top graph indicates where a predator differed significantly from the other predators in their tendency to attack that prey. The * between the graphs indicate instances where the number of marked predators significantly over or under estimated the number of actual feeding events. Ants and *D. signatus* were not used in the analysis (see text).

Marked predators.

Predators differed significantly in their baseline absorbance (their absorbance readings before being exposed to a marker), and in their likelihood of obtaining the mark after eating or encountering marked prey. The differences in baseline absorbance could lead to biases in field collected predators, and could complicate studies on predator-prey interactions at the community level. However, the effect of the baseline absorbance readings probably has little effect on the final absorbance of the marked predator, especially when considering the degree to which predators differ in their ability to acquire the marker by consuming marked prey. Spiders, whose baseline absorbance readings were low, had significantly higher absorbance readings after consuming marked prey than beetles, whose baseline absorbance readings were high. Perhaps a more pragmatic approach would be to adopt a conservative critical absorbance level by calculating the mean baseline absorbance readings from all predators, add four times the standard deviation rather than the usual three times the standard deviation (Hagler & Naranjo 2004; Mansfield *et al.*

2008) and then use a simple multiplicative correction factor to accommodate differences between plates (Jones *et al.* 2006). This conservative approach would enable all predators in the community to be tested without the need to conduct prior tests to determine species-specific critical absorbances for every predator species that may be collected.

The difference between the predator groups in their tendency to acquire the protein marker could be caused by differences in their digestive processes, or feeding behaviour. The spiders in this study all lunge at and grab their prey before wrapping it up and feeding. Bugs such as *N. kinbergii* stab their prey, while beetles *H. variegata* and *D. bellulus* move over their prey and start chewing. The amount of handling of prey by spiders prior to feeding may explain their greater tendency to acquire marks.

The difference between predators in their tendency to acquire the protein marker was reflected in the discrepancies between the percentage of predators marked and the percentage of predators that fed on different prey. In particular, *Cheiracanthium* were over represented and *N. kinbergii* were under represented when marked predators were directly compared to predators which actually ate prey. Overcoming such a bias is difficult, and would be common to all external marking techniques. One solution to the problem of cross-contamination is to mark prey internally by rearing them on an artificial diet that includes the protein marker (Hagler & Miller 2002). Internal marking would be possible for *H. armigera* but artificial diets for the mirid and aphid species used in this study have not been developed.

The discrepancy between the marked and fed predators was enhanced by the high proportion of predators which became marked without feeding on the prey. Ants were a striking example of this as they didn't feed, but they did move around and made contact with the prey item in the Petri dish. The artificial nature of the Petri dishes may have enhanced the likelihood of marking through only encountering the prey in this study. Another explanation may be the sharing of food between ant workers (trophallaxis), which enables a single feeding event by one ant to produce more than one 'marked' individual (Hagler 2006; Morris *et al.* 1998). Nevertheless, if predators can obtain a mark in the field by contacting the prey rather than consuming the prey, then this highlights a potential problem when identifying predators from the field. The problem may be particularly relevant when using egg cards as prey items for predators. If the eggs are marked in situ on the cards, then both the eggs and the card will be marked. Therefore in field studies, care must be taken to only release marked prey (and not marked substrate such as cards), as predators could obtain the mark from walking over the substrate rather than consuming the prey.

These results show that sandwich ELISAs to detect the presence of exotic protein markers are very sensitive and are an effective way of identifying potential predators of marked prey. However caution must be taken when comparing predators from different phylogenetic groups which use different foraging methods. In addition, some predators became marked without feeding on the prey, indicating that care must be taken to ensure that only the target prey are marked and released into the field to minimise incidental marking of predators. Nevertheless, the technique has the potential to reveal cryptic predator/ prey interactions and expose predators of target pests. As such it is a useful tool in the conservation biological control of pests.

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SESSION 16

CAPACITY BUILDING THROUGH ACTION LEARNING IN REGION WIDE BIOLOGICAL CONTROL

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ABSTRACT.

Biological control has long been advocated as a component of sustainable management strategies for endemic and alien invasive pests, particularly in developing countries. Successful implementation of such programs requires significant training of personnel and investment in infrastructure (e.g. appropriate rearing facilities). In addition to the direct benefits of a given program other advantages can include the institutionalization of responsible approaches to biological control and preparedness and the capacity to transfer skills to other pest problems. Failure to adequately invest in the development of human capital may not affect short term successes but misses an opportunity for longer term benefits to accrue. Papers in the session variously consider how the scientific and institutional capacity in biological control can be built through engagement of biological control practitioners in participatory research while strategies are developed and implemented. Three of the papers describe how endemic or introduced biological control agents are incorporated into integrated management strategies for agricultural pests through participatory research in which action learning cycles are employed to modify and improve specific approaches. A fourth paper describes region wide capacity building for the development and implementation of a classical biological control program while the final paper reports on the changing capacity of a network of regional biological control institutions due to changes in investment strategies and priorities. The session highlights the importance of participatory research to build capacity in parallel with the development and implementation of biological control programs but demonstrates that capacity can soon be lost without adequate support and investment.

DEALING WITH EXTANT OR IMMINENT THREATS: A CARIBBEAN EXPERIENCE WITH BUILDING CAPACITY FOR CLASSICAL BIOLOGICAL CONTROL

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ABSTRACT.

The advent of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), in the Caribbean during the mid to late 1990s, brought into sharp focus the serious nature of alien invasive pests on a region-wide scale. The pest posed a serious threat to many countries which had a limited capacity or experience to deal with such a problem. As the invasion wave spread, countries began to realize that it was only a matter of time before they became infested. Fortunately, it was realized early that classical biological control offered a long-term management solution for dealing with the pest. However, as a region, few countries had the capacity to implement classical biological control. This paper describes the capacity building efforts with particular reference on knowledge dissemination and application. We examine the specific challenges and opportunities presented by the hibiscus mealybug, and its impact on regional capacity to deal with similar problems.

INTRODUCTION.

The Caribbean has a long history of classical biological control, but prior to the 1990s, this was largely associated with what was then the West Indian Station of the, Commonwealth Institute of Biological Control, now CABI Caribbean and Latin America. Much of the work in the English-speaking Caribbean prior to 1982 has been well documented by Cock (1985). The period from the 1980s until the advent of the pink hibiscus mealybug (PHMB), *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) in the mid 1990s, saw little action. The earliest report of the PHMB was from Grenada where specimens were positively identified in 1994 (Kairo *et al.* 2000). By this time, pest populations had grown to phenomenal levels and the insect was attacking a broad range of plants. Fortunately, it was realized early that biological control offered the sustainable long term solution that was being sought, but immediate emergency measures included application of pesticides and mechanical removal. Threatened by such a devastating pest, Caribbean countries faced a range of challenges, but perhaps most importantly, was the limited capacity (human, physical and financial) or experience of implementing biological control. This paper explores the capacity building efforts with particular reference to knowledge transfer and application. We examine the specific challenges and opportunities presented by the hibiscus mealybug, and its impact on regional capacity to deal with similar problems.

COMPLEXITY OF KNOWLEDGE NEEDS.

With a host range of more than 200 plants, including crop, ornamental and forest species, the pest caught the attention of entire island populations. Indeed, it was difficult for populations in affected countries to remain unaware of the seriousness and impact of the pest. In Trinidad and Tobago for instance, PHMB attracted no less than four editorials in two of the leading national dailies during the period August 1995 to October 1996 (Dolly, 1996). The stakeholder base therefore included a broad range of entities such as: individual homeowners, farmers, traders, input suppliers, consumers, traveling public, government technocrats and policy makers. All these entities needed some level of knowledge about the pest, but these needs varied considerably in terms of the type of details required. Table 1, summarizes the main types of knowledge needed by different target groups of the population. It also shows that these needs were dynamic and depended on whether or not a country was infested, and if so, at what stage it was in the implementation of the management program. For this purpose, we have identified four temporal categories:

1. Pre-infestation stage: Activities geared towards prevention and early detection of the pest.
2. Early stages of infestation: Emergency control activities involving mainly chemical and mechanical control, and sometimes quarantine measures.
3. Intermediate stage of infestation: Initiation of the implementation of biological control, emergency control measures still in place.
4. Advanced stage of infestation: Countries fully implementing biological control.

APPROACHES TO KNOWLEDGE DISSEMINATION.

As summarized in Table 1, the complexity of knowledge needs varied depending on the stage of implementation of biological control and the target group. Specific knowledge transfer approaches were required in order to maximize learning for particular circumstances. Thus knowledge transfer activities ranged from programs targeting the entire population to narrowly focused training activities directed towards one or a few individuals holding critical positions (Table 2). For instance in non-infested countries where the focus was on prevention or early detection, the greatest emphasis was placed on training the plant protection and quarantine personnel and the general public to know what actions to take. Thus for instance the public would understand why movement of plant products was necessarily restricted and hence cooperate with authorities. The other important dimension was to train the public to recognize the pest so that they could report suspected incipient infestations. It is notable that the public played a critical role in spotting the very first infestations of PHMB, rather than through the elaborate government-sponsored monitoring efforts. Once a country became infested, the emphasis changed to emergency control. For most of the countries which were infested during the initial wave of spread of the pest (1994-97), emergency control usually involved chemical and mechanical approaches, popularly referred to as spray, cut and burn (McCommie, 1999). However, this approach posed a serious challenge once a country began implementing biological control. At this point it was imperative for the public to understand the importance of protecting release sites.

Table 1. Knowledge needs by different sectors of the population at different stages of the infestation-control continuum.

Status of Country	Target Group	Knowledge Need
Not infested.	Traveling and general public, traders (export and import), smugglers.	Recognition of the pest. Actions to prevent accidental introduction of the pest. Government regulations e.g. with respect to trade.
	Policy makers, plant protection and quarantine authorities.	All above. Options for control in order to develop contingency plans.
Infested, emergency control.	General public.	Options available. Government led actions and what to do, or not to do. Recognizing natural enemies.
	Policy makers, plant protection authorities.	Options for control. Requirements for the implementation of biological control. Government policy on aspects such as trade.
	Input suppliers, pest control contractors.	Options for control (chemicals/application).
Infested, emergency control, initiation of biological control. Biological control.	Public.	Safety of natural enemies. How to protect natural enemies. Redistribution of natural enemies.
	Plant protection and quarantine authorities and other relevant government technocrats and policy makers.	Review of natural enemy dossiers. Procurement, culturing, release and monitoring of natural enemies. Exporting natural enemies.

Because of the need to reach a very broad population, the approach here focused mainly on mass media outlets such as television, radio and newspapers, supplemented with specific outreach to communities where releases were being made. Based on the experiences with PHMB in the Caribbean the success of such an approach depended very much on having:

- Clearly defined purpose and goals and recognizing that these would vary depending on the audience.

- Development of consistent learning goals and recognizing that these could be temporal, changing as the program evolved.
- Early identification of potential barriers to learning, which could be social, cultural or economic and factoring these in the program.
- Development of a suite of approaches or channels for knowledge and information transfer and customizing these to specific target audience.

Table 2. A summary of training approaches used during implementation of a management program for the pink hibiscus mealybug in the Caribbean.

Characteristics of Audience	Training Approaches	Effectiveness
Large population e.g. general public.	Public awareness and engagement campaigns including use of: posters and fact sheets, newspapers (articles and advertisements), television, radio, telephone hotlines.	Variable but difficult to assess.
Smaller well defined groups such as specific communities, farmers, traders, school children.	Community meetings and school programs which were largely participatory.	Effective.
Groups requiring technical knowledge: government technicians (plant protection and quarantine, research and extension), policy makers, regional and international agencies, other researchers.	Workshops, study visits and conferences.	Effective
Government technicians (research and extension) and, other researchers.	Internships, study visits and graduate training.	Very effective.

IMPLEMENTATION OF BIOLOGICAL CONTROL.

From the outset, there was already a substantial knowledge on natural enemies of PHMB from Egypt and India which rapidly speeded up the exploratory process (Moursi, 1948; Ghose, 1972; Mani, 1989; Kairo *et al.* 2000). Only a few regional/international agencies were involved in exploratory activities and these included: CABI, Indian Agricultural Research Institute (IARI) and United States Department of Agriculture (USDA). Therefore there was no need for developing local capacity in exploration. Against this background this paper addresses knowledge dissemination and application issues related to the subsequent components of the biological control effort namely: introduction, multiplication, release and monitoring of natural enemies. Based on the differences in capacity, Caribbean countries took

different approaches or processes to the implementation of biological control for PHMB, and on this basis may be categorized loosely into three groups as follows:

- Category 1: Countries which introduced natural enemies from an outside source for local multiplication prior to release.
- Category 2: Countries which introduced natural enemies from an outside source for direct field release.
- Category 3: A combination of categories 1 and 2.

The majority of countries would fall in category 3 although the extent for which they adopted a particular strategy varied considerably. All countries however needed some degree of specific training in order to implement the biological control programs. The training took several forms:

Intensive short term lecture/practical based training.

The nature and length of short term training varied considerably, from programs tailored for specific national needs, to regional training courses and technology transfer workshops. CABI organized at least two regional training workshops in Trinidad with support from the FAO and the European Union. USDA APHIS, OIRSA and CABI organized one technology transfer workshop for Central American countries, in Belize. USDA APHIS also organized another technology transfer workshop for US territories in the Virgin Island.

Internships.

Internships which varied in length from 1-6 weeks. CABI provided internships to technicians from national programs at its laboratories in UK and Trinidad. These were particularly useful for countries establishing programs for rearing natural enemies and varied in length from a one week to six months. Once national programs, established their own programs, these also provided additional opportunities for internships for others.

Experiential training.

Experiential training provided by consultants working directly with national counterparts. Several agencies provided consultants to support national programs. CABI and FAO played a critical role in this area. Other agencies which provided support included, Caribbean Agricultural, Research and Development Institute (CARDI) through a scientist seconded from IARI, USDA Animal and Plant Health Inspection Service and the Commonwealth Secretariat. It is notable however, that as Caribbean countries gained experience, they also provided consultancy support to newly infested countries under the FAO Technical Cooperation among Developing Countries (TCDC) program. This was indeed a true measure of some of the success with consultants coming from Guyana, Grenada and Trinidad and Tobago.

Symposia, workshops, program meetings and seminars.

Several symposia, workshops, meetings were organized at the regional level and these provided an opportunity for discussing ideas or reviewing progress of existing programs.

ESTABLISHING AN ENABLING REGULATORY ENVIRONMENT FOR THE IMPLEMENTATION OF CLASSICAL BIOLOGICAL CONTROL.

One of the significant developments during the early 1990s was the development of the International Code of Conduct for the Import and Release of Exotic Biological Control Agents (Greathead, 1997; IPPC, 1996). The Code was formally endorsed in 1995 as the third International Standard for Phytosanitary Measures (ISPM No. 3) under the International Plant Protection Convention. The initial biological control programs in Caribbean were funded by the Food and Agriculture Organization (FAO) and it was agreed at the outset that ISPM No. 3 would be followed. This was a new experience for all the countries in the region which did not have national mechanisms governing introduction of biological control agents or independent procedures. Indeed, for many countries, plant protection legislation was outdated or non-existent. The biological control effort against the PHMB was therefore a challenge but it also provided an opportunity for countries to establish formalized processes for the introduction of biological control agents. Kairo *et al.* (2003) discuss these benefits and challenges faced by countries during implementation of the standard, and provide examples of how subsequent biological control efforts have benefited. Through an experiential learning process, countries were able to gain knowledge and experience in implementation of the code. ISPM No. 3 has since been revised and renamed, Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and other Beneficial Organisms (IPPC, 2005). It is anticipated that the experiences gained by countries will continue to enhance safe and responsible implementation of biological control.

DEVELOPING CAPACITY IN TAXONOMY.

The advent of PHMB in the Caribbean, emphasized the importance of taxonomy, not only for biological control but for plant protection in general. CARINET, the Bisystematics network of the Caribbean was the first LOOP of BioNET International established in 1996 with the objective to provide countries of the sub-region with the capability in biosystematics needed to achieve their national objectives in sustainable agriculture, biodiversity and the wise use of environmental resources. Established right in the middle of the spread of PHMB, it was easier to highlight the need and importance of biosystematics support. From the "Needs Assessment Survey of the Biosystematic Resources available in the Caribbean" undertaken by CARINET it was obvious there was a serious dearth of trained personnel to service the taxonomic needs of the region. Two short term training regional workshops were mounted specifically focused on Hemiptera:

- Identification of Mealy Bugs and Whiteflies important to the Caribbean region
- Identification of Scale Insects and their Natural enemies

CARINET also facilitated in-depth training of one regional technician in the taxonomy of Sternorrhyncha which then led to the publication of an identification manual of mealybugs of the Caribbean (Watson and Chandler, 2000).

CARINET has organized several other courses focused on important taxa and as the emergence of a number of new has followed PHMB, it is clear this is an area that will require continued attention. In 2003, CARINET also established CariPestNet (<http://pets.groups.yahoo.com/group/caripestnet/>) an email based service maintained by volunteers to provide quarantine and plant protection information to the Caribbean. CariPestNet is essentially a cost free question and answer service through which regional & international experts answer queries received from the Caribbean region. This may include providing preliminary image based identification of problem species. It has a current membership of 168 and continues to receive a steady traffic. It is anticipated that such internet based networks will play an increasingly important role in knowledge transfer and application in the future.

CONCLUSIONS.

The advent of the PHMB in the Caribbean was frightening to most countries but has served to raise the awareness about the risks posed by invasive species. There is no doubt that it raised the success achieved has raised the profile of biological control. Several countries have developed the physical and human capacity for the implementation of biological control as evidenced by the implementation of programs against other target pests such as the citrus blackfly in Trinidad (White *et al.* 2005). Another important benefit has been the institutionalization of responsible implementation of biological control. Clearly, this is an important area that will require continued attention, but progress is being made. Many different international, regional and national agencies worked together to develop effective knowledge transfer and application mechanisms, which served to highlight the importance of partnerships. The establishment of South-South cooperative linkages was also a significant milestone.

DISCLAIMER.

The views expressed here are solely those of the authors and do not reflect the official position or otherwise of any of the institutions mentioned.

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INCREASED MAIZE PRODUCTION THROUGH CAPACITY BUILDING OF BIOLOGICAL CONTROL PRACTITIONERS IN DPR KOREA

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ABSTRACT.

Maize is one of the most important crops in DPR Korea, but maize production is facing extensive problems because of severe damage inflicted by agricultural pests, particularly the Asian corn borer. Field studies conducted in 2005 and 2006 at three cooperative farms to evaluate the potential of inundative releases of *Trichogramma ostrinae* wasps for Asian corn borer management have shown that the number of Asian corn borer larvae and larval tunnel lengths were significantly reduced in release plots by 63.5% and 64.1% respectively. In contrast, maize yield was significantly increased by 28.2% (fresh weight) in *T. ostrinae* release plots compared to non-release plots. To make use of this potential, four *Trichogramma* rearing facilities have been implemented and personnel responsible for managing the facility and rearing the wasps, have been trained intensively. Strict quality control measures were implemented in these local rearing facilities and results indicate that important parameters of *Trichogramma* quality such as parasitism rate, emergence rate, sex ratio and wing deformity are largely in line with international standards. Furthermore, training of personnel at a cooperative farm level was conducted regarding *Trichogramma* release techniques, including timing of releases based on pest monitoring, as well as impact assessment. We conclude that *T. ostrinae* augmentation based on locally produced wasps can substantially increase DPR Korean maize production, thereby enhancing sustainable agriculture and food security.

INTRODUCTION.

Maize, *Zea mays* (L.) (Poaceae), is one of the most important cereal crops in DPR Korea (DPRK). In 2007, the total area of planted maize had reached over 495,000 ha, which is almost 20% of the total arable land of the country. It is an important source of calories and protein for human nutrition. In DPRK, maize is processed by milling to give a number of intermediary products such as flour and meal. These materials, in turn are used to produce a large number of foods including noodles, cakes and bread. The by-products of milling include the maize germ and the seed-coat. The former is used as a source of high quality edible oil, which is particularly scarce in DPRK. The crop is also extremely important as winter fodder for

animals. Availability of maize for draught animals is closely related to their nutritional status and therefore, their work efficiency in terms of soil preparation in spring. Thus, maize has an indirect effect on the overall productivity of the agricultural land.

Since the 1970s, maize production in DPRK has been facing extensive problems because of damage inflicted by agricultural pests including the Asian corn borer *Ostrinia furnacalis* Guenée (Lepidoptera: Pyralidae), a widespread moth that attacks maize throughout Asia (Nafus & Schreiner 1991). Yield losses of grain maize due to this particular pest typically range from 10 to 30%, depending on the year and location, but some extremely high damage levels of up to 80% can occur in DPRK. To control Asian corn borer, broad-spectrum synthetic chemical pesticides are applied at cooperative farms during the first generation of the pest, though these direct plant protection products are not always available. Chemical pesticides are extremely difficult to apply during the pest's second generation, because by this time the maize plants are too tall for conventional treatment and sophisticated machinery would be required. Therefore, biological control using *Trichogramma* wasps have been considered by the government. During the 1980s, the Ministry of Agriculture (MoA) and the scientific advisory services of the Academy of Agricultural Sciences (AAS) were prompted to develop a nation-wide programme on *Trichogramma* application. Thus, the first *Trichogramma* mass rearing facility was established in the mid-1980s under the responsibility of MoA and annual releases of this biological control agent were made in nearly every maize-growing county. A total of 230,000 ha of maize were treated annually with *Trichogramma* between 1980 and the early 1990s. The biological control programme, however, started to fail in 1993 and only 50,000 ha were treated that year. The programme finally collapsed in 1995 when food shortage was severe and all available resources had to be redirected for human nutrition. As a consequence, barley, wheat or maize, required for the mass production of *Trichogramma*, were no longer available. In addition, several technical problems had occurred with the *Trichogramma* production procedure, mostly involving rearing contamination by mites. The impact of the *Trichogramma* release programme at a co-operative farm level was further reduced due to a lack of knowledge by the farmers involved. In fact, they were not comfortable with the application technique and also, farmers working in the rearing units were finding the production of natural enemies to be time consuming and not always efficient.

Since 2004, the Centre for Agricultural Bioscience International (CABI) has been working with the DPRK to re-establish an effective integrated pest management (IPM) programme for Asian corn borer control in maize, based on inundative releases of *Trichogramma ostrinae* Pang and Chen (Hymenoptera: Trichogrammatidae) to improve maize production and thereby enhance sustainable agriculture and food security within the country (Zhang *et al.* 2006, 2007). The major aims of this current project were to: (1) re-establish an effective and localized mass production technique for *T. ostrinae*; (2) test the efficiency of *T. ostrinae* field releases; and (3) re-establish *Trichogramma* rearing facilities at the county level with sustainable mass production and high quality standards. Capacity building of DPR Korean biological control practitioners on *Trichogramma* production, quality control and release technology has also been a major aim throughout the implementation of project activities.

MATERIALS AND METHODS.

Efficiency of *Trichogramma* Releases.

In 2005 and 2006, field experiments were conducted to evaluate the effectiveness of *T. ostriniae* releases in maize fields located at the three project cooperative farms (Namsam experimental station, Wongyo and Sokgyo cooperative farms). Eight maize plots per location were randomly assigned to either the *T. ostriniae* release treatment or the control treatment where no wasps were released. Plot size was approximately 0.25 ha (50 m × 50 m). Plots were separated from each other by at least 200 m to minimize the chance of *T. ostriniae* dispersal from release plots to control plots. In all the plots, the maize variety Chongyun No 4 was planted at a density of 54,000 plants per ha (Zhang *et al.* 2006, 2007).

The *T. ostriniae* egg cards used for the study (Fig. 1) were produced with seed culture of the wasps from DPRK, by Hengshui Tianyi Biocontrol Company (HTBC) in China. Releases were made when the first host egg mass was observed in the field. Three releases were done against the 1st generation of Asian corn borer (beginning of June to mid -July) with 3-5 day intervals between releases. Another two releases were conducted against the 2nd generation of Asian corn borer (end of July to beginning of September), again after the first egg masses of that generation were observed. In each plot of 0.25 ha, 42 egg cards, each with approximately 900-1000 parasitized eggs, were set out, resulting in a density of about 150,000 parasitoids per ha for each release.

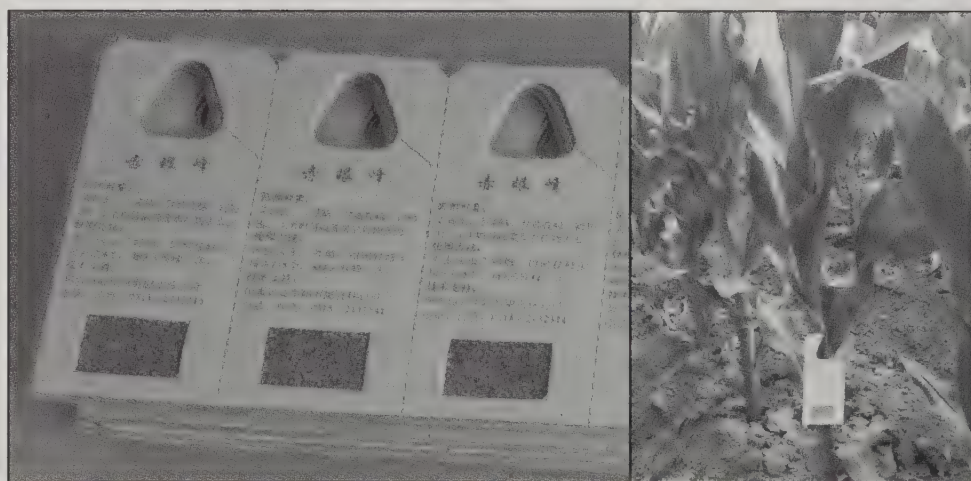


Fig. 1. *Trichogramma ostriniae* egg cards (left) and their application (right) in the maize fields at experimental plots in 2005 and 2006 in DPR Korea.

In order to evaluate the effectiveness of *T. ostriniae* releases, larval damage was assessed in September at or shortly prior to harvest. In each plot, 100 plants were sampled in a diagonal line within the plot (10 plants × 10 rows) with rows chosen randomly. The number of holes caused by Asian corn borer larvae, per plant, were counted. Each plant was dissected, and the larval tunnel lengths were measured. In addition, maize yield was estimated in each plot by weighing the wet

mass of all the husked maize ears collected from the plants in a Pyong (1 Pyong = 3.3 m²) at five randomly selected locations. Calculations were made for the proportion of decrease or increase of 1st and 2nd generation Asian corn borer larvae, the tunnel length, and the fresh yield in *Trichogramma* release plots compared to control plots. Data from all three cooperative farms and all plots, both years and both generations was used to analyse the effect of *Trichogramma* releases on plant damage and maize yield. Data was log transformed if needed to match assumptions of parametric statistics. Subsequently, full factorial ANOVA was used to compare the results between release and control plots.

Establishment of *Trichogramma* Rearing Facilities.

In a first step, an Experimental *Trichogramma* Rearing Facility (ETRF) was established in 2005 at the Plant Protection Institute (PPI) of AAS in Pyongyang as a resource centre for stock culture rearing, small-scale production *Trichogramma* wasps for research, and as a basis for further expansion of the technology and the associated knowledge needed to run the facilities within the country (Zhang *et al.* 2006). A Maize IPM Focus Group (MIFG) with four research entomologists from AAS-PPI was established to implement project activities on a daily basis. Technical equipment for mass production of both *T. ostriniae* and the host moth, *Sitotroga cerealella* (Oliv.) (Lepidoptera: Gelechiidae), was introduced from HTBC of China for pilot testing at ETRF (Zhang *et al.* 2007).

In a second step, the establishment of a *Trichogramma* Rearing Facility (CTRF) at a county level was conducted to allow production of the parasitoids close to farms where releases were planned (Zhang *et al.* 2007). Two locations, Mangyongdae District of Pyongyang City Province and Koksan County of North Hwanghae Province were selected for introducing Chinese production technology and scaling up of production capacity to cover 700 ha of maize fields each. A production team including a manager, a technician and workers (6 staff per facility) was mobilized by MoA and County Farm Management Board. Technical equipment shipped from China was installed at each location.

To increase long-term sustainability of *Trichogramma* production, a third step aimed to adapt the Chinese mass production technology according to local conditions such as electricity and the availability of other inputs. The major aim was to achieve the same output and quality of *Trichogramma* wasps by using the newly developed local CTRF design. A *Trichogramma* Innovation Focus Group (TIFG) was established at the Central Plant Protection Station (CPPS) of MoA, consisting of *Trichogramma* rearing experts and machinery-making engineers. The objective was to develop a local CTRF design, equipped with locally produced technical equipment that considered the experiences during the 1980s in DPRK. The SunAn *Trichogramma* Rearing Facility in the SunAn District of Pyongyang City was selected at the end of 2007 to be the test site for implementation of the local CTRF design.

Quality Control.

Quality control measurements of *Trichogramma* produced at each rearing facility were conducted in 2008, before field releases to control the 2nd generation of Asian corn borer. A sample of 100 – 150 parasitized *Trichogramma* eggs were randomly chosen from the production and glued to a 10 mm x 10 mm piece of graph

paper to measure parasitism. Ten such paper cards were placed individually into glass vials with secured lids and held at 25-28 °C and 60 – 80RH. After adult emergence, the number of emerged wasps, the number of females, the number of males and the number of adults with deformed wings were counted. Proportional data was arcsin-sqrt transformed for statistical analysis. Single-factor ANOVA was used to compare the quality control measurements taken among the four rearing facilities.

Participatory Training on Field Releases and Production.

Capacity building through knowledge transfer to DPR Korean partners was of high importance for the success of the project. A participatory training and research (PTR) approach was used throughout the project, taking the special situation in DPRK into consideration (Zhang *et al.* 2006, 2007; Grossrieder *et al.* 2005, 2008). Partners, especially the MIFG members were actively involved in the project planning process. Training of Trainers (TOT) for the MIFG, conducted by international consultants, was on the *Trichogramma* field releases and impact evaluation, *Trichogramma* mass production with Chinese technology and measurements of quality control etc. Subsequently, the MIFG provided training on these topics to farm extension officers at the project cooperative farms (one extension officer for each cooperative farm), as well as two rearing technicians at the CTRF (one technician for each CTRF). Thereafter, farm extension officers trained work team leaders at their cooperative farms (seven to eight work teams per farm) who further passed the knowledge to the other 50 farmers in their work teams. Rearing technicians, further trained rearing workers at their CTRF (four workers for each CTRF). During this knowledge transfer process, training activities such as lectures, field demonstrations and hands-on experience were made. In addition, CTRF personnel attended two-week training courses focusing on *Trichogramma* mass production technology and business plan development at HTBC in China in 2005, 2006 and 2007, respectively. Finally, comprehensive training material was developed on *Trichogramma* rearing to support knowledge transfer and allow broader dissemination to biological control practitioners across the country.

RESULTS.

Efficiency of *Trichogramma* Releases.

Release of *T. ostriniae* had large effects on the parameters measured. In fact, the number of Asian corn borer larvae (pooled for 1st and 2nd generation) was significantly reduced in release plots by 63.5% ($F_{(1,72)} = 336.0$, $P < 0.001$) while larval tunnel length was reduced by 64.1% ($F_{(1,30)} = 161.8$, $P < 0.001$, see Fig. 2). In contrast, maize yield was significantly increased from 3.1 kg/Pyong to 3.9 kg/Pyong, i.e. by 28.2% on average (fresh weight) in *T. ostriniae* release plots compared to non-release plots ($F_{(1,36)} = 38.67$, $P < 0.001$).

Establishment of *Trichogramma* Rearing Facilities.

ETRF has been successfully functioning since its establishment in 2005. *Trichogramma ostriniae* stock culture, produced at the ETRF, has been delivered to CTRF at Mangyongdae and Koksan (75 g for each CTRF) at the beginning of every production season in March according to schedule. The established ETRF has been

also functioned as the supplier of wasps (around 200 g parasitized eggs in total per field season) for field release at Namsam experimental station and laboratory research, as a source for technical support to CTRF and as a national training center to provide theoretical and hands-on experience to rearing technicians of CTRF on *Trichogramma* production (also see session of Participatory training on field releases and production)

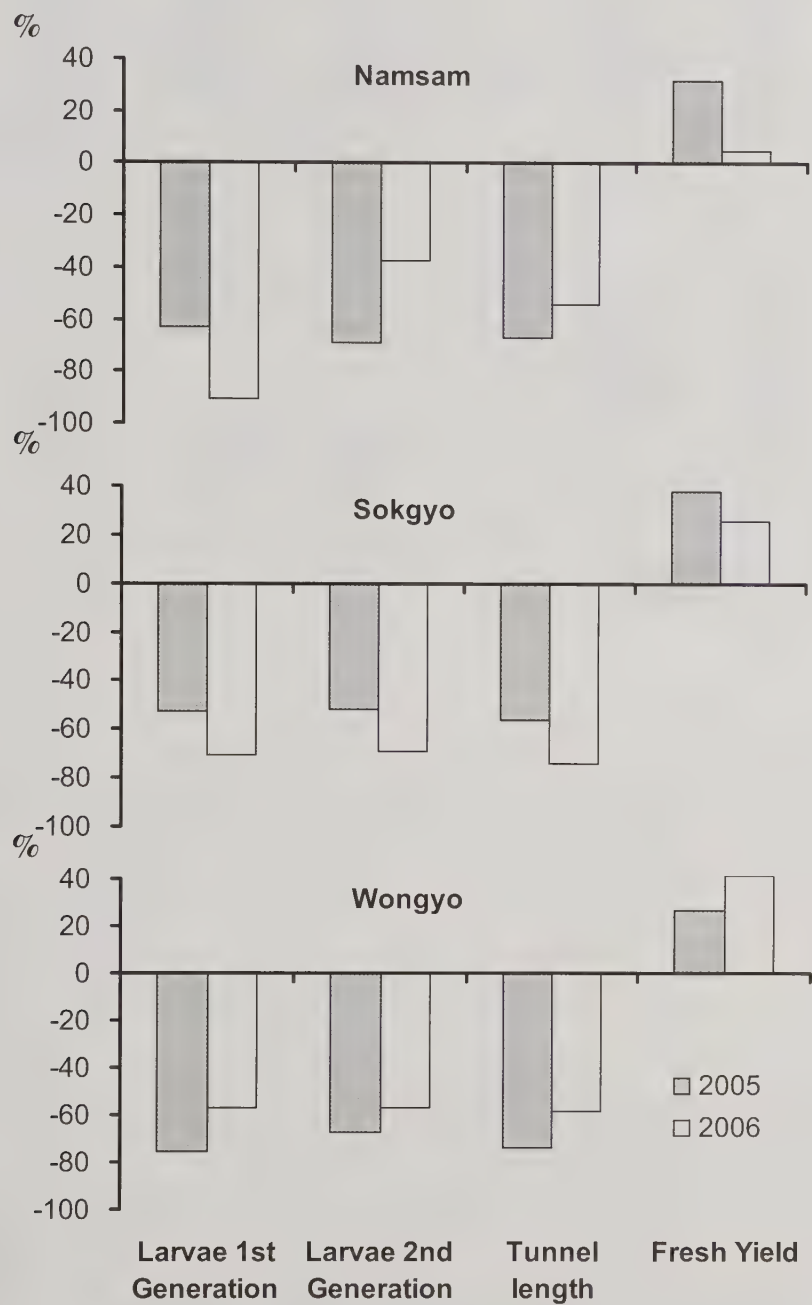


Fig. 2. Percent decrease (-) or increase (+) of Asian corn borer larvae (separated by 1st and 2nd generations), tunnel length and fresh yield in *Trichogramma ostriniae* release plots in comparison to control plots at Namsam experimental station, and Sokgyo and Wongyo cooperative farms in 2005 and 2006.

Since its establishment in early 2006, CTRF Mangyongdae has been progressing step by step with an output of *Trichogramma* products for field release to

cover 64 ha, 350 ha and 700 ha of maize fields in 2006, 2007 and 2008, respectively. Aiming to manage the facility sustainably, a business plan has been developed as a model document for CTRF Mangyongdae with components including organizational structure and terms of references, annual input and output calculations, machinery maintenance and depreciation costs, products distribution plan etc. Also starting in 2006, CTRF Koksan is one year behind the production schedule, i.e. the production was approximately 350 ha in 2008.

The local CTRF design was developed by TIFG with construction design blueprints for all technical equipment. Overall electricity consumption of the local CTRF design was reduced by 66.7% in comparison to the Chinese production system. In addition, the local CTRF design would be able to produce *Trichogramma* successfully with limited access to electricity over time. For example, an oven run by coal is used to sterilize the medium and other rearing materials. Depending on availability, the medium used for rearing *S. cerealella* larvae can be either barley (as in the Chinese production system) or maize grains as in the traditional DPRK rearing system. In consideration of potentially high humidity (up to 90%) in July and August, host moth rearing cages were designed with improved air ventilation and wooden host larvae rearing containers were constructed instead of using stainless steel. Host egg collection and purification machinery are operated manually, therefore without the dependence of electricity. A traditional cold storage room, made from ice was built for overwintering *T. ostriniae* eggs. The local design has been partially tested in SunAn with an output of *Trichogramma* products for field release to cover 200 ha of maize fields in 2008.

Quality Control.

Results of quality control measurement at the four *Trichogramma* rearing facilities are shown in Fig. 3. Parasitism varied between 67.3% at ETRF PPI and 90.9% in CTRF SunAn with highly significant differences among the different rearing facilities (ANOVA; $F = 45.4$; $df = 3,35$; $p < 0.001$; Fig. 3A;). Pairwise comparisons revealed that differences between individual facilities were also highly significant (Tukey's HSD test, all $p < 0.001$) except for PPI-Mangyongdae ($p = 0.395$).

The emergence rate observed was always higher than 90% although differences among the rearing facilities are significant ($F_{(3,31)} = 5.991$, $P = 0.002$; Fig. 3B). Highly significant differences were observed in the sex ratio of production among the different rearing facilities ($F_{(3,36)} = 19.7$, $P < 0.001$; Fig. 3C). Sex ratio at ETRF PPI (69.7%) and CTRF Mangyongdae (77.7%) was significantly higher than at CTRFs SunAn (49.0%) and Koksan (56.7%) ($p < 0.05$). The proportion of healthy females without deformed wings was higher than 93.4% for all the rearing facilities with no significant differences among the different rearing facilities ($F_{(3,36)} = 1.32$, $P = 0.283$; Fig. 3D).

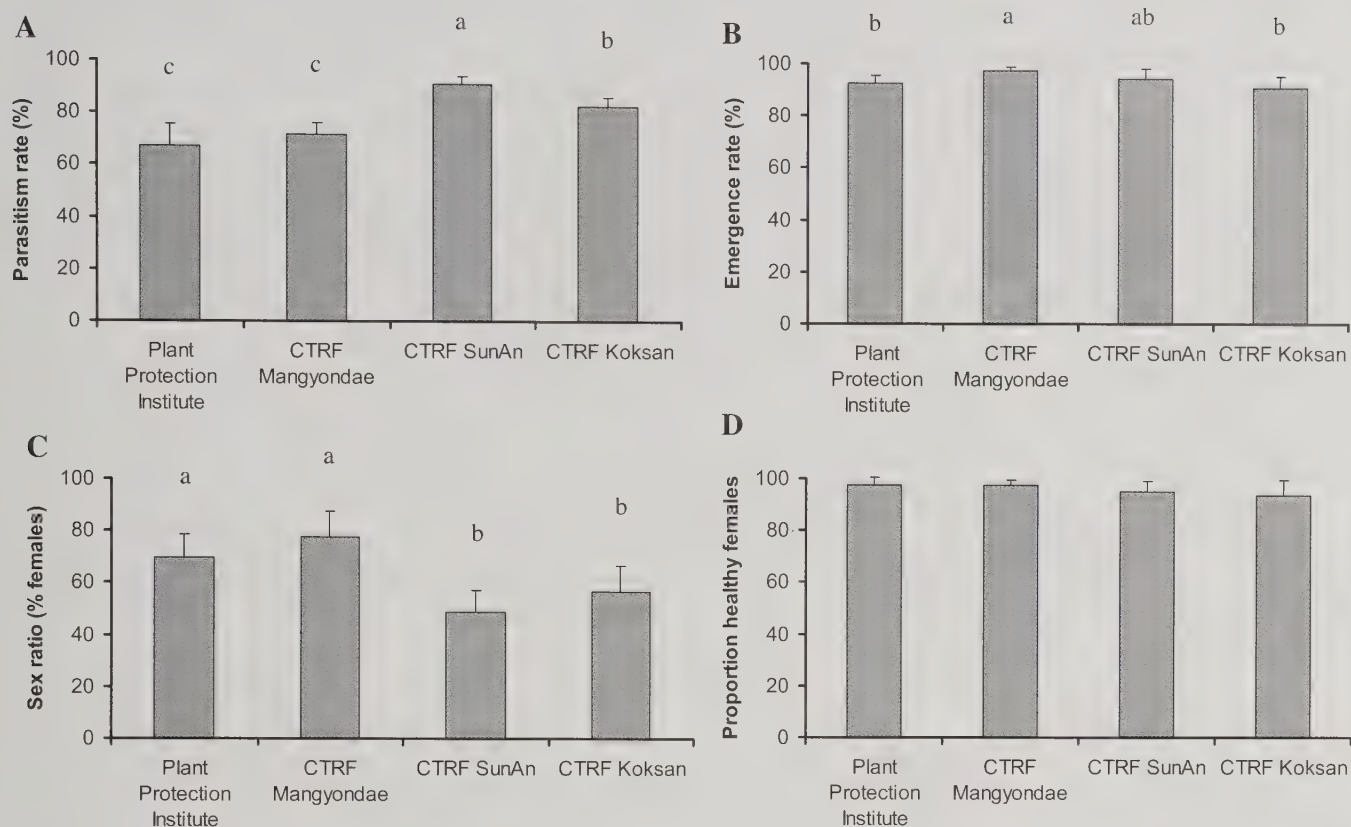


Fig. 3. Quality control measures for *Trichogramma* wasps produced at four *Trichogramma* rearing facilities in DPR Korea: (A) mean parasitism rate, (B) emergence rate, (C) sex ratio, and (D) proportion healthy females. Different letters above the column bar indicates the significant differences at $p < 0.05$.

Participatory Training on Field Releases and Production.

With the PTR approach, four MIFG members were fully trained by international consultants and Chinese *Trichogramma* experts on *Trichogramma* field releases and mass production and they became master trainers during the project implementation. The 13 technical officers and rearing technicians from CPPS and CTRF were trained by MIFG on *Trichogramma* mass production at the ETRF of AAS-PPI, from which, three rearing technicians from CTRF provided further training to another 12 workers at their rearing facilities. At the cooperative farm level, three extension officers were trained by MIFG on *Trichogramma* releases, who then transferred this knowledge to 22 work team leaders and 1,100 farmers in their work teams. Two AAS-PPI scientists and four CPPS and CTRF managers were also trained on business plan development of CTRF at HTBC in China. A direct effect of the training was, for the first time, the development of a business plan for CTRF Mangyongdae on *Trichogramma* production in DPRK. In addition, three AAS-PPI scientists, one technical officer of CPPS, three rearing technicians and 12 workers of CTRF were trained on quality control measurement of *Trichogramma* products.

As an important tool for training activities, a *Trichogramma* Rearing Manual has been developed as a didactic training material within the project. In 2009, 1,000 Korean copies of each manual will be distributed to a larger audience of biological control practitioners across the country.

The use of pesticides for Asian corn borer control in DPRK is not a very promising option due to problems with application against the 2nd pest generation, farmer health concerns and irregular availability. Therefore biological control by using *T. ostriniae* wasps as a component of an IPM strategy, is one of the remaining tools available for maintaining profitable maize production in DPRK. In fact, the use of *Trichogramma* is considered a sustainable option for corn borer pest control in many countries of the world (Li 1994; Smith 1996) and inundative releases of *Trichogramma* spp. to control Asian corn borer have been successful in a number of Asian countries, especially China (Li 1994, Wang *et al.* 2005). Like in China, *T. ostriniae* was found to be the dominant species recovered from field collected Asian corn borer eggs at project cooperative farms in DPRK (Zhang *et al.* 2006). In the present study, inundative releases of *T. ostriniae* (5 releases, in total 750,000 wasps per ha) have substantially suppressed Asian corn borer larval damage, while at the same time maize yield has increased. Except for Namsam in 2006, where yield increase is less obvious, differences in yield increase was between 25.9% and 41.6%, thus rather consistent over farms and years. These results indicate that *T. ostriniae* releases provide a promising IPM tool in DPRK maize fields. The use *T. ostriniae*, will not only significantly improve maize production but will also have a significant positive environmental impact, because negative short- and long-term effects of synthetic chemical pesticides on farmer health as well as on the environment will be avoided. A number of studies have clearly shown that beneficial insects will be harmed less by *Trichogramma* wasps compared to insecticides (Babendreier *et al.* 2003a, b).

For each programme involving inundative biological control agents, quality issues are of the highest relevance and concern (van Lenteren 2003). Although quality control was conducted only before the first release to control 2nd generation Asian corn borer, i.e. after eight production cycles, the results presented here for the four rearing facilities reflect high standards of *T. ostriniae* quality. Except for a lower parasitism rate at ETRF PPI and CTRF Mangyongdae and a noticeable lower sex ratio (% females) at CTRF SunAn and Koksan, the quality of the produced egg cards is in line with international standards (Bigler *et al.* 1987; Lundgren & Heimpel 2003). Based on promising results regarding quality of *T. ostriniae* products and the capacity of covering 700 ha of maize fields, the local CTRF design will be further tested and potentially improved in 2009 at another four different locations in maize production areas of DPRK, taking into consideration a business plan.

Best results with *Trichogramma* worldwide in the past have been based on trained personnel who provide not only the product alone but also extension support to the end-users in the field (Smith 1996). In the current project, three extension officers and 1,100 farmers at project cooperation farms have been trained to release *Trichogramma* wasps in their maize fields. Training has also been focusing, to some extent, on rearing personnel at CTRF. An important basis of any further training exercise will be the recently developed *Trichogramma* rearing manual in Korean

language which will be widely used by the rearing personnel as a guideline for good rearing practices.

With an average of 28.2% yield increase achieved in the present study, it is expected that large-scale application of *T. ostriniae* releases would contribute significantly to stabilize the country's maize production as well as support community efforts to avoid reversion into a food emergency situation. With further support (from the Swiss Agency for Development Cooperation and the European Commission), it is envisaged that the local CTRF design would be multi-replicated at up to 24 different locations across the country in the next three years. At least 72 cooperative farms and 25,200 farmers will benefit from the established CTRFs in the near future by receiving biological control agents for Asian corn borer control and achieving subsequent yield increases. Assuming that the relative yield increase is similar to the current project (28.2%), it is expected that an increase of 1.07 t/ha could be achieved (based on a national average maize yield of 3.79 t/ha in 2005/2006, see FAO 2008). This means that if 24 new facilities are established, and they produce according to the business plan, the annual maize production of DPRK would increase by 17,955 t (16,800 ha x 1.07 t per ha yield increase).

CONCLUSIONS.

With the success of the maize IPM project as shown above, the DPR Korean government is currently planning to implement this approach to over 200'000 ha in the near future. In preparation for this, a national workshop was held in 2008 at the established CTRF SunAn with 75 *Trichogramma* experts from the whole country to exchange knowledge, to evaluate the traditional rearing method and local CTRF design, and to spread the adapted advanced rearing technology. Altogether, a number of positive developments may eventually enable large scale IPM in maize to become a reality, including: (1) relevant stakeholders clearly gained beneficial experience during the current project; (2) training of farmers will be improved; (3) a Farmers' Manual for Maize IPM will be developed; (4) model CTRFs together with a business plan and a rearing protocol, including quality control methods are available; and (5) the recent adoption of cabbage IPM guidelines as part of the national crop protection policy (Grossrieder *et al.* 2008). These developments clearly indicate the possibility of IPM becoming institutionalized at the national level in DPRK.

ACKNOWLEDGEMENTS.

We thank the farmers, extension officers and chief engineers of Sokgyo and Wongyo cooperative farms and Namsam experimental station for their support. We also thank County Farm Management Boards of Koksan and SunAn District for their support in providing rooms and logistics towards establishment of County *Trichogramma* Rearing Facilities. The work presented in this paper has been funded by the Swiss Agency for Development and Cooperation, Bern, Switzerland.

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INSTITUTIONAL CAPACITY BUILDING IN APPLIED ENTOMOLOGY THROUGH COLLABORATIVE RESEARCH IN DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA

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ABSTRACT.

Brassica crops represent more than half of total vegetable production in DPRK and two major lepidopteron pests, *Plutella xylostella* and *Pieris rapae*, pose the principal constraints to production. On co-operative farms pests have been traditionally managed by scheduled application of broad spectrum insecticides with no attempt to conserve or utilize endemic natural enemies. In a collaborative research initiative, scientists from the University of Queensland worked closely with scientists from Sariwon Agricultural University and the Korean Academy of Agricultural Sciences, to build capacity in the fundamental aspects of integrated pest management and its implementation. The aim of the collaborative research was to identify and study the natural enemy complexes attacking *P. xylostella* and *P. rapae* on co-operative farms. Initial training was delivered via reciprocal country visits and stakeholder workshops. The entomological expertise of DPRK scientists was consolidated through a series of supervised field experiments, which compared the impact of prevailing pest management practices with pest-threshold based interventions of *Bacillus thuringiensis* (Bt), designed to conserve natural enemy populations. The Bt-natural enemy strategy increased crop yields by up to 56% and enhanced the impact of both parasitoids and predators on pest populations. On conclusion of the formal project in 2006, DPRK scientists successfully performed experiments to investigate the effects of management scale on the efficacy of natural enemies, demonstrating considerable increased capacity in integrated pest management since the start of the project. Further development of the strategy has since been constrained by the lack of available resources in DPRK.

INTRODUCTION.

Since the mid 1990s, the Democratic People's Republic of Korea (DPRK) has faced a critical shortfall in national food production. Poor soil fertility, a series of catastrophic floods and lack of access to essential fertilizers following the collapse of the Soviet Union (Michalk & Mueller, 2003) culminated in a series of devastating famines in the mid-late 1990s (Bhatia & Thorne-Lyman, 2002). Increased international food aid and improved harvests between 2002 and 2005 improved the situation (Watts, 2005) but food security remains poor and the country suffered further famine in 2008.

In DPRK all agricultural production is prescribed by the government through a system of co-operative farms (co-farms) (Fig. 1).Vegetable co-farms are 100–500 ha in area and each supports a residential workforce of 1000–2000 people which is divided into a series of work-teams led by a manager and a chief engineer. Each work-team is responsible for the cultivation of a range of crops including *Brassica* vegetables, cucumber, maize, onion, potato, pepper, pumpkin and tomato. *Brassica* crops represent more than half of the approximately 200,000 ha of vegetables which are grown on co-farms annually; the major crops are “white cabbage”, *Brassica oleracea* L. (Brassicaceae), which is principally grown in spring- early summer, and Chinese cabbage, *B. campestris* L. (Brassicaceae) which is primarily grown in late summer- autumn. Insect pests represent a significant constraint to the production of both crops. The key pests of white cabbage crops are the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) and the small white butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae) and their combined impact can reduce yields by as much as 40% (Grossrieder *et al.* 2005). Under current co-farm standard practices, insect pest management in *Brassica* crops relies entirely on the application of broad spectrum insecticides such as deltamethrin and phosphamide. Economic conditions currently limit the accessibility of insecticides in the DPRK and ecologically based pest management strategies which maximize the impact of available natural enemies and eliminate unnecessary insecticide applications are the only practical means to ensure the sustainable production of essential *Brassica* crops. Effective IPM programs for the management of *Brassica* pests have been implemented in many regions of the world (Ivey & Johnson, 1998; Furlong *et al.* 2004). However, before a specific strategy can be designed for a particular crop system the key natural enemy species attacking the key pest species must be identified and relationships between pest, natural enemy and crop phenologies must be understood.

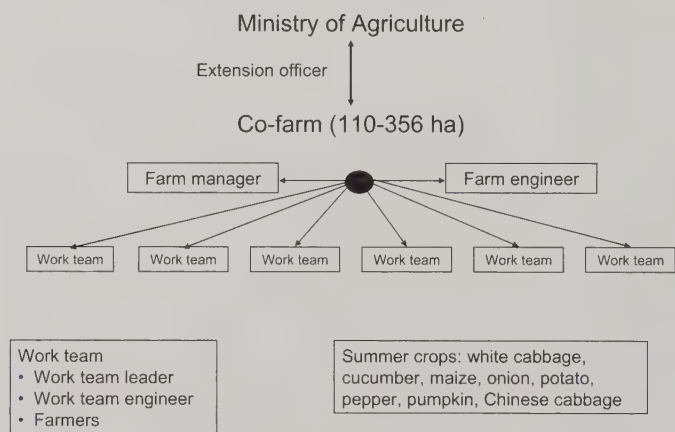


Fig. 1. The management structure of co-operative farms in Democratic People’s Republic of Korea. Vegetable production and all pest management extension at the co-farm, county and province level is under the direct control of the Ministry of Agriculture in accordance with the National Plan.

Isolation and prevailing economic circumstances in DPRK have left the country critically short of capacity and the infrastructure required to conduct the research and development necessary for the implementation of national pest management strategies. This paper describes the approach taken to increase institutional capacity in applied entomology in DPRK through basic collaborative research and the subsequent design and implementation of an IPM program for the

management of the key insect pests of *Brassica* crops. Researchers at the University of Queensland worked closely with researchers at the key research institutions of the Plant Protection Institute, Korean Academy of Agriculture Sciences (PPI-KAAS), and Sariwon Agricultural University (SAU). Laboratories at each institution were equipped with air conditioned insect rearing rooms, incubators, microscopes and basic entomological equipment. These institutions have been assigned by the Ministry of Agriculture to undertake research on the implementation of IPM for vegetable crops in accordance with the National Plan.

MATERIALS AND METHODS.

Collaborative Research 2003-2005.

In the design phase of the project aims and objectives were devised and agreed upon via a series of reciprocal country visits by DPRK and University of Queensland scientists. The overall objective of the project was to build capacity in applied entomology in DPRK by conducting collaborative research to develop an integrated pest management (IPM) program for insect pests of *Brassica* crops. The approach was based on strategic interventions with *Bacillus thuringiensis* var. *aizawai* (GC-91, Wuhan Kernel Bio-pesticide Co., China) and the conservation and utilization of endemic natural enemies of the key pest species. To achieve this, the collaborative research activities addressed the following series of sub-objectives:

1. Design and execution of surveys for the key insect pests of *Brassica* crops and their natural enemies in south western regions of DPRK
2. Demonstration of the principles of IPM in DPRK
3. Assessment of the impact of natural enemies on key pests in DPRK
4. Derivation and testing of economic/ intervention thresholds based on the pest complex
5. Development sampling, data analysis and decision making skills of DPRK scientists

Experimental sites. Experiments and surveys were conducted in summer white cabbage (*Brassica oleracea* var. *capitata* L. cv 6-21) at Oryu co-farm (356 ha) on the outskirts of Pyongyang and at Tae Sung co-farm (110 ha) on the outskirts of Sariwon, 70kms south of Pyongyang.

Brassica pest and natural enemy surveys. The phenology *P. xylostella* and *P. rapae* in summer white cabbage crops was studied by stratified random sampling of 60 plants in 1ha fields at each site every three to four days. Selected plants were thoroughly searched and the stage and number of all insects present on each was recorded. Sampling commenced within one week of seedlings being transplanted to the field in mid April and continued until crop harvest in late June or early July. In order to determine the apparent rates of parasitism by larval and pupal parasitoids, fourth instar and pupae of *P. xylostella* and fourth and fifth instar and pupae of *P. rapae* were collected and returned to the laboratory for individual rearing (see Furlong *et al.* 2008). Epigeal predators in the agro-ecosystems were sampled by setting a 60m grid of 16 pitfall traps (8 cm diameter) in two fields at each site in June 2004 and 2005. Traps remained in the ground for two weeks and were emptied every two to four days, contents were immediately sorted and identified to species where possible (Furlong *et al.* 2008).

Formulation and testing of preliminary IPM strategy. A preliminary IPM strategy based on the conservation of endemic natural enemies and threshold based interventions with Bt was devised and field tested in 1-2 ha fields of white cabbage at each of the co-operating co-farms. On each farm the strategy was compared to current co-farm standard practice, prophylactic application of deltamethrin, in a nearby field of a similar size. For the IPM strategy, a series of dynamic intervention thresholds was developed based on a “standard insect” (SI); 1 SI=1 *P. rapae* larva or 5 *P. xylostella* larvae. Thresholds based on crop growth stage were set as follows: pre-cupping stage= 1SI/ plant; cupping stage 0.5 SI/ plant; heading stage 4 SI/plant. In both IPM and co-farm practice fields, 60 plants were sampled every 3-4 days as previously described and population counts were immediately converted to SIs. When relevant thresholds were exceeded in the IPM fields Bt was applied within 24 h, deltamethrin intervention in co-farm practice fields was under the control of the farm manager and engineer.

The impact of natural enemies on *P. xylostella* populations. In 2005 the impact of natural enemies on *P. xylostella* populations was tested under the two different pest management regimes at each co-farm by using fine nylon net cages to exclude natural enemies from laboratory derived cohorts of *P. xylostella* (n=25 eggs/plant). Recovery (proportion of initial cohort recovered) and survival (proportion of initial cohort eclosing as adult moths) rates were compared with recovery and survival rates of identical cohorts developing on similarly caged plants to which natural enemies had access (detailed methods in Furlong et al., 2008). Confidence intervals were constructed using a modified version of Abbott’s formula incorporating an estimate of the variance of the control (natural enemy exclusion) mean (Rosenheim & Hoy 1989). The impact of the endemic predator complex was estimated by comparing recovery rates and the impact of the combined action of the entire natural enemy complex was estimated by comparing survival rates.

Collaborative Research 2006.

At a planning meeting in Hangzhou, China in November 2006 the potential impact of the management unit on the success of an IPM program was discussed and plans made to test the effect of substantially increasing the area of the IPM management unit on natural enemy abundance and crop yield. Between December 2005 and August 2006, no contact between Australian and DPRK collaborators was possible; the project formally ended at the end of June 2006. Between April and July 2006 DPRK scientists set up large scale experiments at Tae Sung and Oryu co-farms to test the impact of management unit scale on key natural enemies, pest abundance and crop yield. On each co-farm, four work teams were organized to grow their white cabbage crop in close proximity to each other and create a contiguous crop of approximately 6ha; this was then managed according to the IPM strategy previously described. A fifth work team cultivated a smaller white cabbage crop (approximately 1ha) approximately 1000m away from the contiguous crop in an area surrounded by other vegetable crops; this crop was also managed according to the IPM strategy. A sixth work team cultivated another white cabbage crop (approximately 1ha) at least 1000m away from the contiguous crop in an area surrounded by other vegetable crops; this crop was managed using standard co-farm practices. In each crop on each farm plants were sampled every three to four days, pest densities were recorded and, where appropriate, transformed into SI values in order to make Bt

intervention decisions. Each week appropriately aged *P. xylsotella* and *P. rapae* larvae and pupae were collected and reared in the laboratory to estimate apparent rates of parasitism. Insecticide input into the co-farm practice and IPM plots was recorded and when the crops matured they were harvested and yield measured.

RESULTS.

Collaborative Research 2003-2005.

In 2003 several visits to DPRK were made by University of Queensland scientists and a further visit was made at the beginning of the summer cabbage growing season in May 2004. During the course of the project DPRK scientists were not able to visit Australia and no visits to DPRK by Australian scientist were possible after May 2004. In 2005 the DPRK and Australian project teams held a week-long meeting in Hangzhou, China in order to plan and coordinate future research activities; this was the last meeting between the scientists from the two countries during the project.

Brassica pest and natural enemy surveys. *Plutella xylsotella* larvae were attacked by *Cotesia vestalis* Haliday (Hymenoptera: Braconidae) and *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae) and pupae were attacked by *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae). *Cotesia vestalis* was recovered from both sites in both years of the surveys; the *C. vestalis* apparent parasitism rate was usually <20% but did reach 57% at one site (Table 1). Similarly, *D. collaris* was recovered from one site in 2004 but from both sites in 2005; its apparent parasitism rate was <10% at both sites in 2005 but reached 46% at Tae Sung in 2004 (Table 1). *Oomyzus sokolowskii* was not recovered from either site in 2004 and only at extremely low levels from both sites in 2005 (Table 1). In 2004 *C. glomerata* L. and *C. rubecula* Marshall (Hymenoptera: Braconidae) were recovered from *P. rapae* larvae at both sites and combined parasitism rates reached 42%, *P. puparum* was only recovered from Tae Sung, and parasitism rates reached 33% (Table 1). In pitfall traps the predatory epigeal fauna was dominated by spiders from the family Lycosidae (dominant species *Lycosa t-insignata*) and beetles from the family Carabidae (Coleoptera) (dominant species *Chlaenius naeviger* (Morawitz); *C. pallipes* (Gebler); *Calathus helensis* (Schaller); *Epomis nigricans* (Wiedemann); *Stomis prognathus* (Bates) and *Diplocheila zeelandica* (Redtenbacher)).

Formulation and testing of preliminary IPM strategy. In 2004 *P. xylsotella* reached extremely damaging densities of 33 and 23 larvae per plant in crops managed by co-farm practice at Tae Sung and Oryu co-farms, respectively. In crops managed by the IPM strategy, *P. xylsotella* populations were maintained at densities 50-66% lower on each co-farm. In 2005 *P. xylsotella* densities were lower than in 2004 and populations peaked at 12 and 7 larvae per plant in crops managed by co-farm practice at Tae Sung and Oryu; as in 2004, *P. xylsotella* densities were maintained at 66-74% lower densities in corresponding crops managed by the IPM strategy. *Pieris rapae* populations were generally much lower than *P. xylsotella* populations at both sites in each year of the study. Densities rarely exceeded 1-2 larvae per plant and there was little difference between *P. rapae* population densities in crops managed by co-farm practice and the IPM strategy. However, *P. rapae* was always the first pest recorded in each crop and this, combined with its greater overall weighting in the threshold intervention strategy, often led to insecticidal interventions.

During the study, the co-farm managed crops were treated with deltamethrin significantly more often, an average of 4.2 (± 0.2) times per crop, than crops managed by the IPM strategy, an average of 2.7 (± 0.2) times per crop ($F_{(1,8)} = 24.5$, $P = 0.001$). The IPM strategy increased crop yield (mean weight of marketable cabbage heads, estimated by randomly selecting 10 plants from each of the three sampling blocks per field) by 8-56% when compared to co-farm practice.

Table 1. Parasitoids reared from larvae and pupae of *P. xylostella* and *P. rapae* collected at Tae Sung and Oryu co-farms May- July 2004 and May – July 2005.

Pest ¹	Collection site	Year	Number collected		Parasitoids reared	Apparent % parasitism range ²
			Larvae	Pupae		
<i>P. xylostella</i>	Tae Sung	2004	1112	836	<i>C. vestalis</i>	0-57
					<i>D. collaris</i>	2-46
	Oryu		724	-	<i>C. vestalis</i>	0-19
	Tae Sung	2005	917	824	<i>C. vestalis</i>	0-19
					<i>O. sokolowskii</i>	0-1
					<i>D. collaris</i>	0-9
	Oryu		957	674	<i>C. vestalis</i>	3-14
					<i>O. sokolowskii</i>	0-1
					<i>D. collaris</i>	0-7
<i>P. rapae</i>	Tae Sung	2004	1125	142	<i>C. glomeratus/</i>	
					<i>C. rubecula</i>	0-42
					<i>P. puparum</i>	8-33
	Oryu		753	-	<i>C. glomeratus/</i>	
					<i>C. rubecula</i>	5-32

¹During surveys maximum *P. rapae* density reached 10.4 (± 1.8) larvae plant⁻¹ and maximum *P. xylostella* density reached 32.6 (± 0.8) larvae plant⁻¹

² Apparent rates of parasitism were calculated from appropriate stages. *P. xylostella* collections: *C. vestalis* rates calculated from late fourth instar larvae; *O. sokolowskii* rates calculated from late fourth instar larvae and pupae; *D. collaris* rates calculated from pupae. *P. rapae* collections: combined *C. glomeratus* and *C. rubecula* rates calculated from fourth and fifth instar larvae; *P. puparum* rates calculated from pupae. See Furlong *et al.* (2008) for full explanation.

The impact of natural enemies on *P. xylostella* populations. At each site estimated predation rates and estimated losses due to the combined action of the natural enemy complex were significantly greater in crops managed by the IPM strategy than in crops managed by co-farm practice (Fig. 2). At both sites, and under both management regimes, predation accounted for most of the measured pest mortality (Fig. 2).

Collaborative Research 2006.

In the large scale experiments conducted at Tae Sung and Oryu co-farms in 2006, *P. xylostella* densities were much lower than those recorded in 2004 and 2005; *P. xylostella* population densities were similar in the 6ha IPM, 1ha IPM and co-farm practice plots (Fig. 3). *Pieris rapae* populations were similar to those in previous

years, 2006 densities rarely exceeded 1-2 larvae per plant and there were no differences between densities in crops managed by the different pest management regimes (Fig. 3).

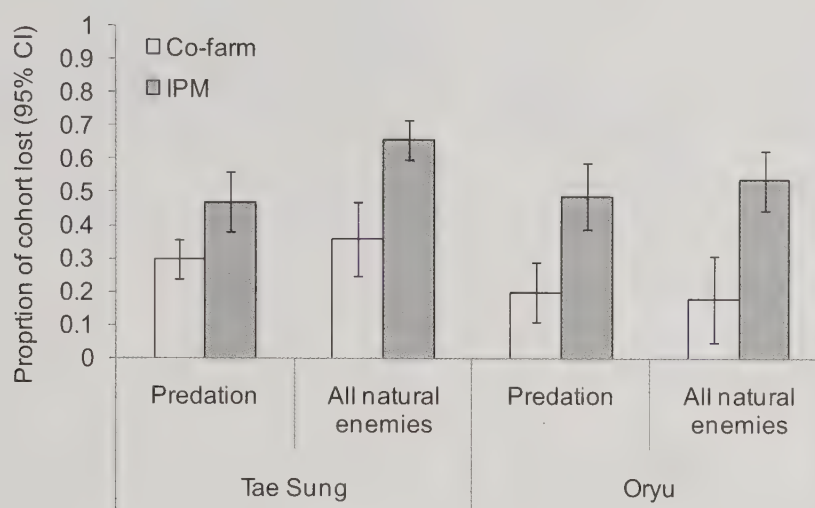


Fig. 2. Estimates (95% CI) of the impact of predators and the combined natural enemy complex on experimental cohorts of *P. xylostella* in crops managed by the IPM strategy and standard co-farm practice at Tae Sung and Oryu co-farms in 2005.

In the first generation of each pest, parasitism rates of *P. xylostella* by *C. vestalis* and parasitism rates of *P. rapae* by *C. glomeratus* and *C. rubecula* were extremely low at both sites (1.5-10%; Fig 4). Based on the overlap of 95% CIs, neither *P. xylostella* parasitism rates nor *P. rapae* parasitism rates were affected by pest management practices at either site in this generation (Fig 4). Between the first and second generations, parasitism of *P. xylostella* by *C. vestalis* increased significantly in the 6ha IPM crops at both Tae Sung and Oryu co-farms (Figs. 4a and 4b). During the same period, *C. vestalis* parasitism rates did not significantly increase in crops managed by co-farm standard practice at either site while the *C. vestalis* parasitism rate increased in the 1ha IPM crop at Tae Sung but not at Oryu co-farm (Figs. 4 a and 4b). Similarly, between the first and second generations, parasitism of *P. rapae* by *C. glomeratus* and *C. rubecula* increased significantly in the 6ha IPM crops at both co-farms (Figs. 4c and 4d). *Pieris rapae* parasitism rates in the 1ha IPM crops also increased between generations at both sites (Fig 4c and 4d) but *P. rapae* parasitism rates, although higher in the 2nd generation, did not significantly increase in crops managed by co-farm practice (Fig. 4c and 4d).

Crop yield was not affected by pest management practice at either co-farm (Table 3). At Oryu co-farm, three insecticide applications were applied to all crops but at Tae Sung, the co-farm practice crop received more insecticide applications than either of the IPM managed crops (Table 3).

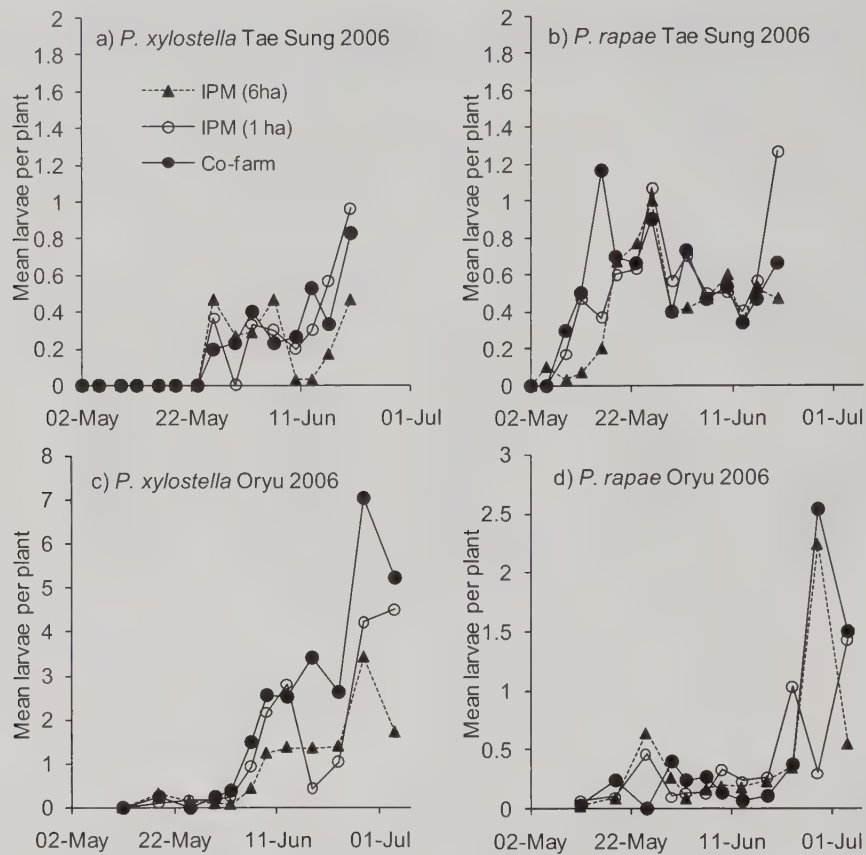


Fig. 3. Population densities of *P. xylostella* and *P. rapae* in crops managed by IPM (6ha contiguous fields (n= 120 plants sampled) or smaller 1 ha fields (n=30 plants sampled)) or co-farm standard practice (n=30 plants sampled) at Tae Sung and Oryu co-farms in 2006.

Table 3. Crop yield under different pest management regimes and the number of insecticide applications applied to each crop at Tae Sung and Oryu co-farms in 2006.

Co-farm	Management regime	Mean cabbage head weight (kg) (\pm SE) ¹	Number of insecticide applications ²
Tae Sung	IPM (6ha)	0.97 (\pm 0.25)	2
	IPM (1ha)	1.08 (\pm 0.28)	1
	Co-farm standard	0.78 (\pm 0.27)	3
Oryu	IPM (6ha)	1.01 (\pm 0.24)	3
	IPM (1ha)	0.96 (\pm 0.23)	3
	Co-farm standard	0.89 (\pm 0.28)	3

¹ There was no significant management regime effect on cabbage crop yield at either co-farm (ANOVA $P>0.05$). ² *Bacillus thuringiensis* was applied to crops in both IPM management regimes, deltamethrin was applied to crops managed by co-farm standard practice.

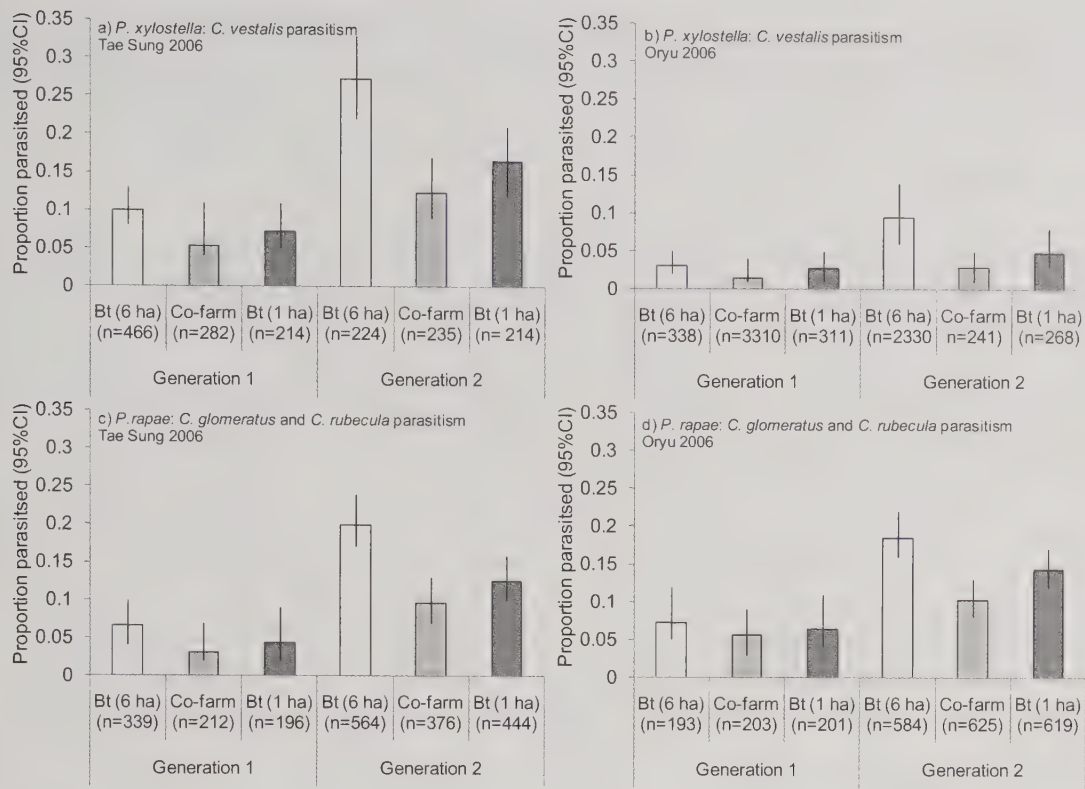


Fig. 4. Apparent parasitism rates of *P. xylostella* and *P. rapae* in white cabbage crops managed by IPM or co-farm practice strategies at Tae Sung and Oryu co-farms 2006.

DISCUSSION.

During the project, circumstances beyond the control of the collaborating scientists restricted the number of possible reciprocal visits and frequent direct contact between research groups was difficult. Nevertheless, with the considerable support of ACIAR's North Asia office, contact was maintained and workshops were convened in China in order to review progress, confront logistical and scientific problems and plan research activities for the coming crop season. Careful project planning and a high degree of commitment by DPRK scientists ensured that the all project sub-objectives were achieved. Pest surveys over successive years demonstrated that *P. rapae* infested crops soon after planting but that infestations by *P. xylotella* often occurred later and were more variable in intensity. It is likely that *P. rapae* is endemic to DPRK whereas *P. xylotella* probably makes annual migrations from more southerly regions (Furlong *et al.* 2008). The major larval parasitoids of *P. rapae* and *P. xylostella* are probably endemic to DPRK (Furlong *et al.* 2008) but make only a minor contribution to pest population suppression under co-farm management practices. Although approaches to conserve natural enemies significantly increased parasitism rates, the contribution of parasitism to pest mortality remained low even when the IPM strategy was adopted; generalist predators had a significantly greater impact on *P. xylostella* populations than either of its major parasitoids. In 2004 and 2005 the intervention thresholds tested as a part of the IPM strategy proved extremely effective and resulted in increased crop yields of up to 56% and the necessity of significantly fewer Bt applications to IPM managed crops than the number of deltamethrin applications recommended for co-farm practice managed crops. Further, the natural enemy complex in crops managed by IPM made

a significantly greater contribution to pest mortality than the natural enemy complex in crops managed by co-farm practice and contributed to the reduced requirement for Bt applications to these crops.

CONCLUSIONS.

The increased capacity in applied entomological skills acquired by collaborating DPRK scientists as a result of the collaborative research is evidenced by the large, unsupervised field experiments conducted in 2006. It is likely that the efficacy of important predators and parasitoids in crops managed by IPM strategy is compromised by the widespread use of broad spectrum insecticides in proximate cabbage and other vegetable crops. In order to test this, the scale of the IPM management unit was increased from 1ha to 6 ha at both Oryu and Tae Sung co-farms and pest populations, the number of insecticidal interventions, pest parasitism rates and crop yields were compared with those in 1ha crops managed by the IPM strategy and co-farm practice at both sites. The experiments showed that increasing the size of the IPM management unit significantly increased pest parasitism rates. However, pest populations were lower in 2006 than in previous years and no significant differences in crop yield was detected between management approaches.

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EVALUATING SCIENTIFIC INSTITUTIONAL CAPACITY FOR BIOLOGICAL CONTROL: A CALIFORNIA STUDY AS A MODEL REGIONAL NETWORK ASSESSMENT

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ABSTRACT.

Assessing scientific institutional capacity investigates the ability of scientists and their organizations to achieve their goals. This can be measured using social science metrics, including publications, funding, scientist-years (SYs), and research projects. We report a 3-year long capacity assessment of the 8 regional institutions engaged in arthropod biological control in California 1962-2006. Biological control in California has a strong reputation, but this reputation was established by researchers at the University of California, which does not currently provide support at historical levels. The most significant consequence of this has been a marked decline in the number of dedicated biological control scientists: entomologists dedicated to applied research without responsibilities for administration or undergraduate teaching. Other factors affecting this decline are: changes in organization of entomology departments; new configurations of entomology faculty positions; and the broader forces restructuring the practice of biological control science itself. The California Department of Food and Agriculture's Biological Control Program has assumed a somewhat greater role, but it cannot compensate for the losses at UC, and it has suffered serious budget cuts as well. The state biological control program hosts scientists dedicated to biological control research and practice. We recommend methods for conducting institutional capacity analysis in other regions.

INTRODUCTION.

Scientometrics is the measurement and analysis of scientific activity. Its methods can be used to assess the capacity of scientific institutions to achieve their goals (Leydesdorff 2001), including agricultural science institutions (Warner 2007). This can be measured using social science metrics, including publications, funding, scientist-years (SYs), and institutional connectivity through networks. Perkins & Garcia (1999) were the first to deploy scientometrics to evaluate biological control institutions. Here we report select findings from a 3-year long study of California's institutions that host scientists conducting biological control of arthropods (Warner *et al.* 2008). Biological control in California has a strong reputation, largely established by researchers at the University of California (UC). Critics such as Jennings (1997) have charged that the UC administration abolished the UC Division of Biological Control under political pressure, but the evidence for this is ambiguous. To date, only anecdote has been used to argue that UC has lost capacity for conducting biological

control research. Our study sought to evaluate trends in research capacity among leading regional institutions by using scientometric data.

MATERIALS AND METHODS.

We adapted the methods of Perkins & Garcia (1999) to focus on one region, California. We gathered 7 types of original data on 8 different institutions, but due to space limitations, we only report 4 types of data on 3 institutions (see Table 1).

Table 1. Data collected on key California biological control institutions

	Scientist positions	Quantitative survey	Qualitative interviews	Targeted pests
University of California entomology faculty	SY	X	X	#
California Department of Food & Agriculture Biological Control Program	SY	X	X	#
Other California universities	SY	X	X	

#=number of targeted pests; SY=scientist year; X=data gathered

We identified all UC entomology faculty members at all 3 campuses for the period 1962 to 2006 and gathered data about their scientific activities to assess their research in biological control relative to other interests (for this paper, nematologists are included in the general category of entomology). We included all full-time faculty and extension specialists assigned to these departments. We excluded emeritus and adjunct (part-time) faculty, and staff research assistants. We excluded faculty for whom dates of employment were missing or ambiguous. This resulted in a population of 246 scientists. We relied on different kinds of data to determine their scientific activities. We devised the following hierarchy of data sources, from most preferable (A) to least preferable (E). If data from source A was unavailable, then we would turn to B, then C, then D. In several cases we examined multiple types of data.

- A. Survey questionnaire querying about the types and numbers of biological control projects and publications. This was possible only for scientists currently on faculty at the 3 departments. The survey of contemporary UC entomology faculty was conducted by email and webpage in May 2007. Of the current 83 faculty, 32 responded, for a response rate of 38.5%.
- B. Curriculum Vitae (CV).
- C. Obituaries. These scientific obituaries, prepared by colleagues or fellow members of a department, provide a summary narrative of the research agenda of the scientist.
- D. Abbreviated CVs, campus catalogues or departmental files.

Of the 246 scientists identified, we gathered sufficient data on 199. Interviews with 8 UC scientists provided historical perspective and interpretation of these records. We evaluated SYs at the California Department of Food & Agriculture (CDFA) Biological Control Program, drawing from annual program reports from 1993 to 2004. We surveyed and interviewed 2 scientists at other universities involved in this field. We analyzed records of university scientists to determine their degree of

involvement in biological control, using the coding system of Table 2. We also coded this biological control work according to taxa targeted (chiefly arthropod; chiefly weed; or, mixed).

Table 2. Coding system for scientific activities

Code	Description
1. Dedicated biological control scientist	These have met one of the following criteria: publishing 2 or more major books on biological control; having >30 publications on this topic; or >30% of > 50 publications.
2. Biological control scientist	This category designates scientists who have devoted a considerable portion of their research to biological control, such as foreign exploration. They have published 4 or more papers with the term "biological control" in their title.
3. Scientist supportive of biological control	This category designated scientists who have done some biological control research, but it has not been the major thrust of their work. Survey entry reports research in biological control, they have but 1-3 publications within the field of biological control. Many scientists working on IPM fall into this category.
4. Scientist not involved in biological control	These have not conducted any measurable biological control research.

We evaluated the number of targeted pests by these institutions. For baseline data we used a list of arthropod pests actively targeted by biological control projects and a list of arthropods proposed as targets in 1992 (Division of Agriculture and Natural Resources, 1992, pages 10-11). These are titled "some insect and mite pests currently targeted by biological control projects using predators and parasitoids in California" (Table 2 on page 10-11), and "a partial list of California insect and mite pests thought to be good candidates for control by importation of natural enemies" (Table 3 on page 11). UC researchers identified the current status of these projects, using the coding system of DeBach (1964) of complete, substantial or partial. With the help of CDFA Biological Control Program scientists, we likewise assessed the arthropods it targeted 1993-2004 as identified in the program's annual reports.

RESULTS.

Of the 199 scientists we were able to code, 72 participated in biological control research (Fig. 1). Entomologists in the UC system peaked slightly above 110 in the mid-1980s, and dropped dramatically during the early 1990s owing to university restructuring. At UC Berkeley, the number of entomology positions dropped from 52 in 1984 to 21 in 1996. Of particular interest are the most active researchers (code 1 and 2). During this study period, UC had 19 dedicated biological control scientists (code 1), and 25 biological control research scientists (code 2), chiefly at the Berkeley and Riverside campuses. The number of UC entomologists coded 1 and 2 rose from the mid-teens in the 1960s to the high teens in the 1970s to the high twenties in the 1980s, but then declined to 17 in 2006. Between 1965 and 1984, the number of entomologists coded 1 fluctuated between 12 and 15; this declined down to 6 in 2006. Of the 19 code 1 scientists, 17 chiefly targeted arthropods, 1 weeds,

and 1 mixed. Of the two other entomologists conducting arthropod biological control research, one is code 2 and one is code 3. Of the 2 biological control scientists working at other universities, 1 is a code 2 and 1 code 3.

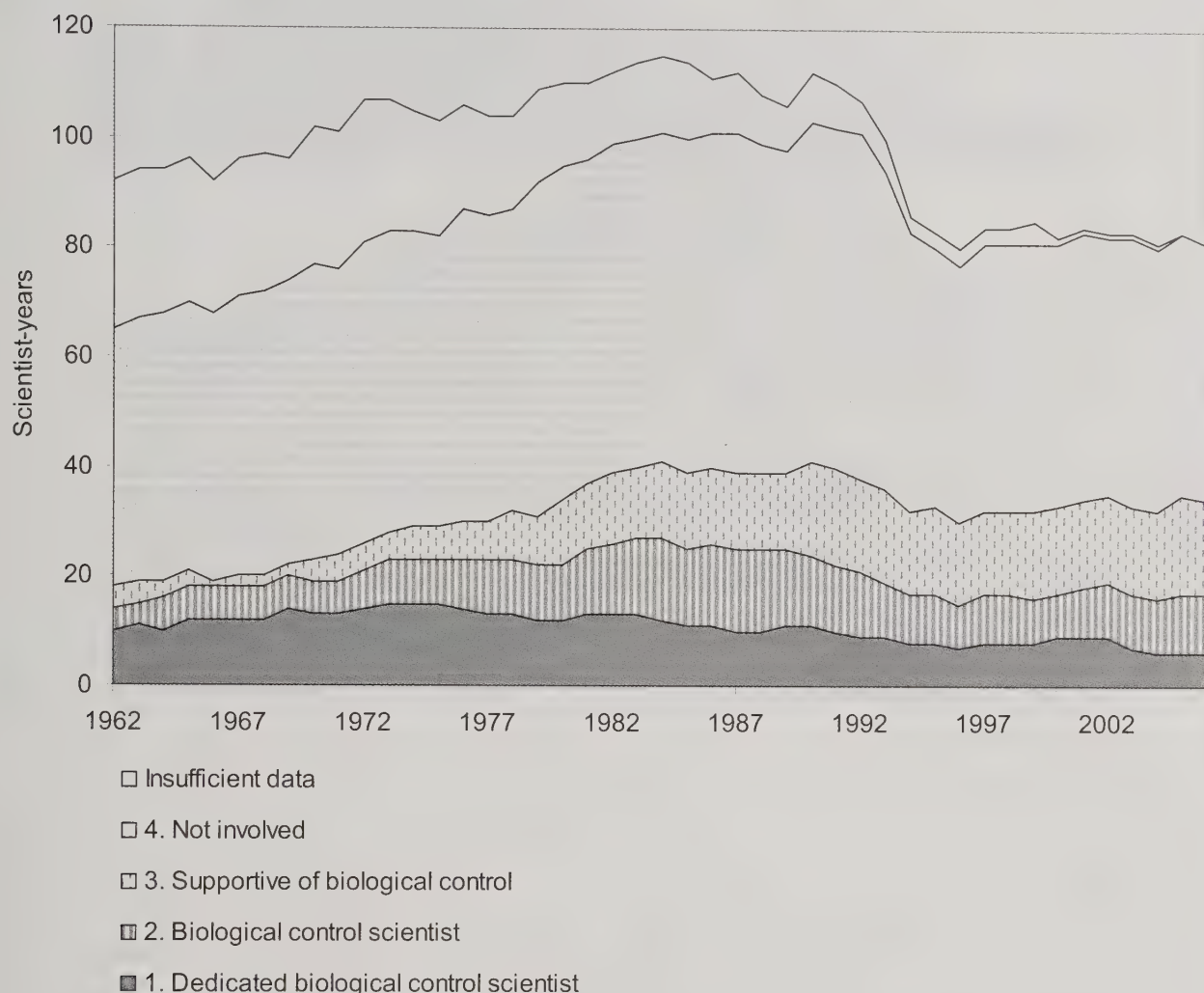


Fig. 1. Scientist-years and their relative investment in biological control at the University of California, 1962-2006.

The CDFA Biological Control Program began in 1974 with 4 SYs and rose as high as 14 in 2001, but has suffered major budget cuts due to state funding since then (Fig. 2). The program is dedicated exclusively to biological control. CDFA scientists have been assigned in equal numbers to arthropod and weed pests.

Of the 43 arthropod pests targeted in 1992, 19 were under some degree of biological control in 2007 (Table 3). For projects with unknown status, we assume no control. Some control was provided in 44% of those arthropods targeted in 1992, although only 9% were complete or substantial. Less than 6% of those “thought to be good candidates” were under any reported biological control in 2007. Even though a list of proposed targets is qualitatively different than pests actively targeted, the difference between 44% of those targeted under some control versus only 6% under any control for those proposed for a target is striking.

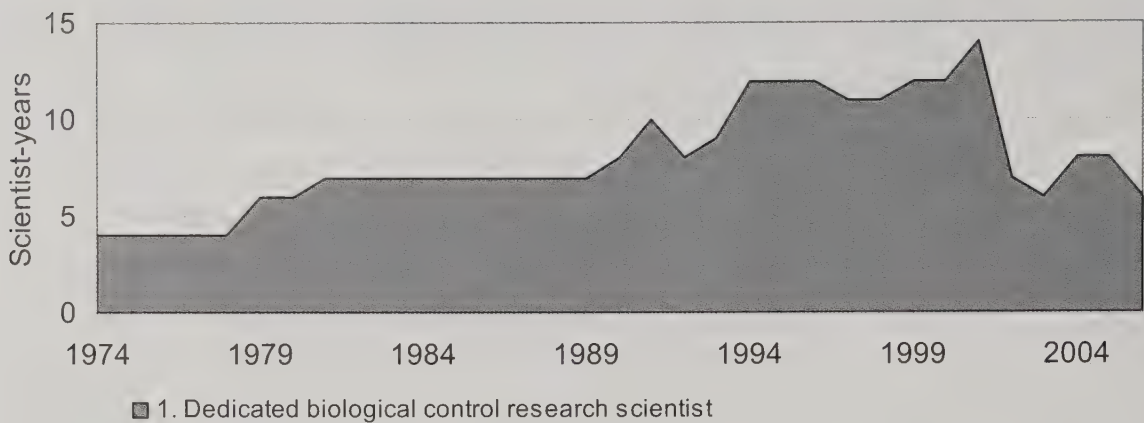


Fig. 2. Scientist-years and relative investment in biological control at the California Department of Food & Agriculture Biological Control Program, 1974-2005.

Table 3. Status as of 2007 of arthropod pests targeted by or proposed for UC biological control efforts in 1992

	Pests targeted by researchers in 1992	Pests “thought to be good candidates” in 1992
Complete control	1	2
Substantial control	3	--
Partial control	15	--
Unsuccessful	18	--
Deemed inappropriate target	--	6
No data	6	26
TOTAL	43	34

Between 1993 and 2004, the CDFA Biological Control program evaluated 24 arthropod pests, achieving some form of biological control with 10 (Table 4). The category “no data” may indicate that the pest project is in the early stages of development, or that the project has been suspended for lack of results or to prioritize other projects. Weed projects are not reported here, even though they constitute half the program’s effort.

Table 4. Status as of arthropod pests considered by CDFA Biological Control Program between 1993 and 2004

	Arthropod species evaluated as targets
Complete control	3
Substantial control	3
Partial control	4
Unsuccessful	-
Deemed inappropriate target	4
No data	10
TOTAL	24

DISCUSSION.

Arthropod biological control in California has a global reputation, but it was not as vigorous in 2007 as it was historically. UC and CDFA are the only institutions of significance hosting biological control research activities targeting arthropods in California. All the data presented indicate a declining institutional capacity to conduct biological control research. The most important declines have been in the number of overall SYs, the proportion of the most active (code 1 and 2) researchers, and the elimination of UC departments dedicated to biological control. The SYs working in this field in UC and CDFA have substantially declined, starting in 1985 and 2001 respectively. The decline of capacity at UC is notable owing to losses of dedicated biological control scientists. In the case of arthropod pests targeted by UC scientists, data also suggest a decline. It is not possible to determine a trend in pests targeted by the CDFA program with this data.

Several caveats are in order. First, these metrics illustrate within-institution trends. Direct comparison of UC and CDFA SYs and targets is not appropriate, however. Entomologists with academic appointments in research universities have different responsibilities and professional incentives than do those working for a dedicated state program. The decline in UC SYs dedicated to biological control is indicative of changes in entomology positions during the study period. Many biological control scientists in the 1960s and 1970s conducted their own foreign exploration and did their own systematics work. With increasing specialization of entomology (as in other biological sciences), few entomologists today are trained in all subfields of the discipline necessary to implement a biological control project. The loss of SYs conducting biological control research at UC are not the result of one single factor. Key influences are: changes in organization of UC entomology departments; new configurations of life science faculty positions; and the broader forces restructuring the practice of biological control science itself. Few new entomology positions are constituted as dedicated to biological control research. The public university-based knowledge system which has served as the anchor for biological control research is not being supported financially as it once was. UC administrators have directed funding and other resources to other academic pursuits over the past 20 years. Few incentives exist for researchers to pursue applied questions, and this poses a much broader set of challenges for California agriculture than merely crop biological control. The elimination of the two UC departments dedicated to biological control marked the loss of more than diminished resources for individual researchers. These departments also provided coordination between different types of researchers, and focus for overall network efforts.

CDFA's program consists exclusively of dedicated biological control scientists, but only half of them work in arthropod control. It began with technicians and only started hiring research scientists in 1991. Its scientists are free from faculty responsibilities, but it has lost more than 50% of its SYs since 2001. It has tried to compensate for the diminished capacity for institutional coordination with the loss of the UC departments, but it still depends upon UC for quarantine space and specialized research. Even though CDFA's program has had many successful projects with a small number of SYs, it draws heavily on UC specialized researchers, and the decline in UC SYs may constrain this program in the near future.

The successful projects in Tables 3 and 4 do not represent all the biological control of arthropod work in California between 1992 and 2006. Many new and serious arthropod pests have invaded California since 1992, and some of these have been targeted by biological control scientists and are under some control. Nonetheless, the number of targeted arthropods and successful projects appears to have declined during this study period. This downward trend is unlikely to be reversed unless additional efforts are made to relate the value of this work to the public (Warner *et al. in press*).

This article demonstrates that assessing institutional capacity for biological control research is possible using scientometric methods. It indicates the importance of investigating the dynamics of change not only in SYs but also in the orientation and activities within SYs. It also points to the importance of studying networks for assessing capacity (Warner, 2007), because as the science of biological control becomes more complex, it requires sharing of specialized knowledge and resources.

ACKNOWLEDGEMENTS.

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TOOLS FOR INCREASING GROWER PARTICIPATION FOR IMPLEMENTING BIOLOGICAL CONTROL

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ABSTRACT.

The Tarnished Plant Bug (TPB), *Lygus lineolaris*, is now the key pest of strawberries in Canada. Current TPB control relies primarily on pesticides which are detrimental to ecosystems. Biological control has not seen much implementation by strawberry growers in Ontario. We developed an approach that encourages growers to participate in defining the strategies based on biological control that are most appropriate for their operation. By presenting to the growers information on the biology of TPB, and how the parasitoid *Peristenus digoneutis* contributes to mortality, capacity is built so that growers can make decisions about how their crop might be best managed using biological control. We then generate data to show how these grower decisions impact on populations of TPB and provide individual reports for each operation. Follow-up meetings with each grower provide a forum for answering questions and deciding on modifications that might be needed to improve the management of TPB. This feedback is used to develop questionnaires and modify protocols for individual operations. Inoculative introductions of the braconid endoparasitoid *Peristenus digoneutis*, which attacks TPB nymphs, have shown increased parasitism levels in strawberries and adjacent refuge areas, which has encouraged growers to continue to participate. Growers are enthusiastic to incorporate biological control into their operation as they understand the economic and environmental benefits.

INTRODUCTION.

The Tarnished Plant Bug (TPB), *Lygus lineolaris* (Palisot) (Hemiptera: Miridae), is now the key pest of strawberries *Fragaria x ananassa* Duchesne (Rosaceae) in Canada. TPB feeding causes the fruit to develop abnormally with cat-facing and apical seediness. TPB has 2 generations and a partial third per year in Ontario. Nymphs of the first TPB generation are the most economically-important on June-bearing cultivars; whereas, the second generation nymphs are the most economically important limiting factor for the production of day-neutral (DN) strawberries. Current TPB control relies primarily on pesticides which are detrimental to ecosystems. Although we have >10 years of data on the biological control of TPB (biology and rearing of parasitoids, parasitism rates, host range, non-target data, comparisons of parasitoids, etc.) (Broadbent et. al 2001, Broadbent et. al 2006, Haye et. al 2005a, Haye et. al 2005b), biological control of this pest has not seen much implementation by strawberry growers in Canada. Factors that contribute to this lack of

implementation may be that we do not have field trial information from a specific crop to show the importance of biological control of TPB and, perhaps more critically, growers have not bought in to this concept and are not well-informed about how to use biological control as part of their crop management strategy. To address these issues we developed a project with the objectives of developing tools to: (1) increase grower participation in developing the use of biological control in their pest management strategy; (2) keep the grower onside during development of the strategy; and (3) provide feedback to the growers so they could be well informed when making decisions.

MATERIALS AND METHODS.

A team consisting of researchers (Agriculture and Agri-Food Canada and CABI Europe, Switzerland) and extension personnel (Ontario Ministry of Agriculture, Food and Rural Affairs) was organized to develop and deliver a program on the biological control of TPB on strawberries in Ontario. The team met to develop an approach that encourages growers to participate in defining the strategies (based on biological control and trap cropping) that are most appropriate for their operation. This approach was based on experience from organic strawberry production in California, expertise in participatory activities within the team, and on the success of the parasitoid *Peristenus digoneutis* Loan (Hymenoptera: Braconidae) establishing and reducing TPB populations in alfalfa in the northeastern USA (Day 1996). Since there are several organic strawberry growers in Ontario a critical decision was to include these growers because they would be the most open to new methods for managing their pest problems. Furthermore, they have a great deal of enthusiasm to produce pesticide-free produce but very little understanding of the role biological control could play.

Increased Grower Participation.

Farmer participatory workshops (FPW) were planned at central locations in southwestern and eastern Ontario and invitations were sent to individual strawberry growers. At each FPW an informal atmosphere was created to ensure that growers were at ease and willing to contribute to the discussions. An overview presentation was made using carefully prepared images to describe the life cycle of TPB, its movement into the strawberry crop, and how the parasitoids interact with TPB and the surrounding habitats (Fig. 1). To achieve a better understanding of the behaviour of the biological control agents, live parasitoids and *Lygus* nymphs were presented to the growers and parasitism was demonstrated in action. In open discussion with all participants, the growers' opinions were solicited with the goal of determining which growers would be most interested in participating in the project. In the spring of the first year, 3 farms in southwestern Ontario and 3 farms in eastern Ontario with growers who were willing to participate in the IPM program were selected to potentially become case studies for the successful implementation of biological control of TPB.

Maintaining Grower Interest.

On-site discussions with farmer participants established background information on the type of operation, strawberry varieties grown, pesticides used (if any) and

frequency, field layout, and surrounding habitats. The advice of these farmers was solicited on how to organize their farms for the field tests that had been planned by the research team. When higher densities of small *Lygus* instars were recognized in the field, growers were asked to assist with the actual releases of parasitoids on their farms to give them a better understanding how to apply biological control agents in the field. At each farm TPB populations and parasitism levels were monitored on a weekly basis from May to September in 2006, 2007 and 2008. Opportunistic interactions with growers during weekly site visits would allow team members to keep the growers up to date on the status of TPB populations and parasitism levels in their crop and non-crop habitats.

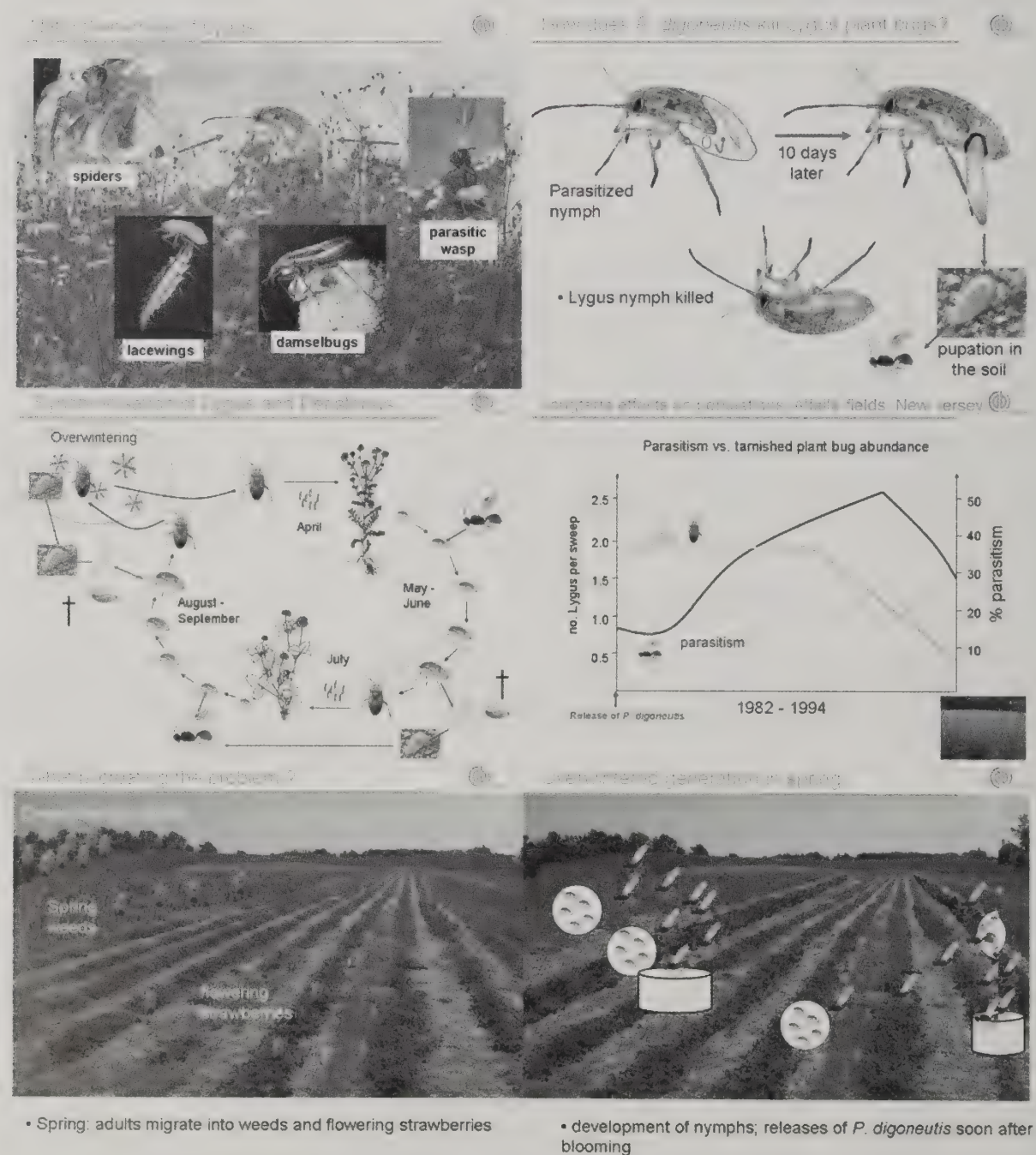


Fig. 1. Representative slides used at farmer participatory workshops to teach growers how biological control of *Lygus* plant bugs could work on their farms.

Tools to Provide Feedback to Growers.

After each growing season, data on strawberry damage, parasitism, and *Lygus* densities were compiled and research team meetings were held during the off season. The focus of these meetings was to analyse the impact of the IPM program at each individual farm and to find solutions to problems encountered. As a result, the initially developed Project Operational Planning document was revised and an updated version was provided to each team member. A 'farmer participant report template' was developed to provide growers with an individualized summary of results on their farm and points for follow up at on-site discussions before the next growing season. It was important to ensure that information provided to individuals compared their farms with those of others but did not identify the other growers.

At the end of the project, a final questionnaire was developed to assess the growers' responses to this project on completion. This document provides the team with feedback on changes in: (1) grower perception of TPB pest status; (2) grower understanding of TPB biology and its natural enemies; (3) pesticide use; and (4) interest for further participation.

RESULTS AND DISCUSSION.

Increased Grower Participation.

By presenting to the growers information on the biology of TPB and how the parasitoid *P. digoneutis* contributes to pest mortality, capacity was built to help growers make decisions about how their crop might be best managed using biological control. Growers became more knowledgeable about the problem and how a biologically-based solution could help. Discussion of constraints for individual growers allowed the team to better design a unique management strategy for each operation. For example, the location, type and size of a trap/refuge crop depended on the land available at each location.

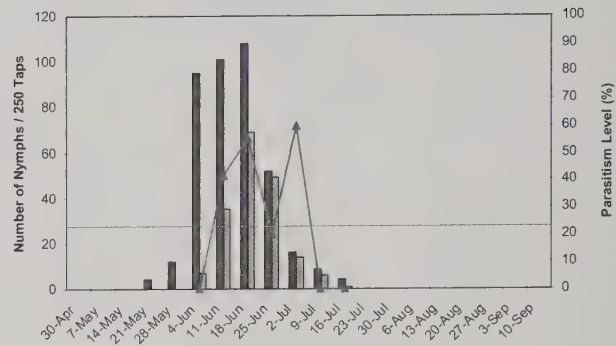
Maintaining Grower Interest.

Generation of on-farm data was used to show how grower decisions impact on populations of TPB (Fig. 2). Inoculative introductions of the braconid endoparasitoid *P. digoneutis*, which attacks TPB nymphs, have shown increased parasitism levels in strawberries and especially in adjacent refuge areas and encouraged growers to continue to participate. The opportunistic interactions with growers proved to be essential for gaining their confidence. Furthermore we were able to provide advice for their consideration about when to cut trap crops. In one case a previously unknown source of TPB infestation was identified and it was recommended that the habitat (clover in this case) be mowed.

Strawberries

The nymph densities were greater in 2008 than 2007. The number of *P. digoneutis* also increased as parasitism levels remained similar from 2007 to 2008.

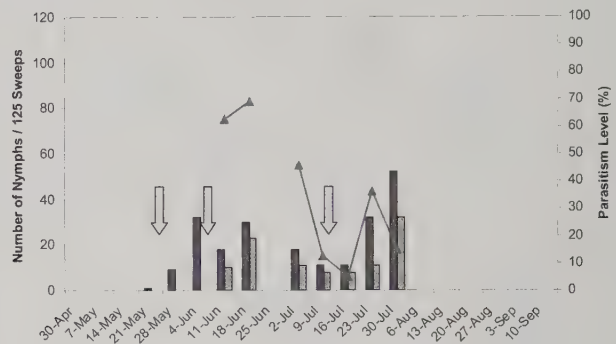
— Indicates threshold



Trap Crop (Clover)

Releases of *P. digoneutis* females

Spikes in parasitism level occurred after *P. digoneutis* releases. Numbers of nymphs in the clover were quite reduced from last year.



Weedy Border

The spikes in parasitism levels indicate the 1st and 2nd generation parasitoids, which also coincided with *P. digoneutis* releases in the trap crop and strawberries.

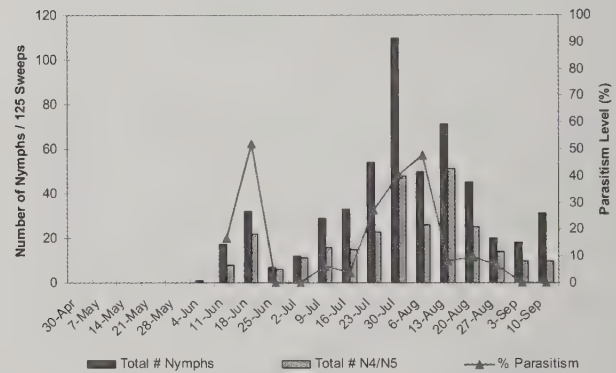


Fig. 2. Representative graphs from grower report: Tarnished plant bug nymph (N4 and N5 instars) densities and parasitism rates in strawberries, clover and weeds at a farm in Centreville, Eastern Ontario 2008.

Tools to Provide Feedback to Growers.

The field data, presented to each grower in an individual report provided an excellent entry for informal discussion by the team. Follow up on-site meetings with each grower provided a forum for answering their questions and deciding on modifications that might be needed to improve the management of TPB for individual operations. For example, it was after this grower input that the original rating scale for strawberry damage was revised.

Three growers did not apply any pesticides and another farm substantially reduced pesticide input in 2008 compared with previous years. All participating

growers were enthusiastic to incorporate biological control into their operation as they understood the economic and environmental benefits. Farmers also realized that the release of the biological control agent, *P. digoneutis*, is expected to contribute to the long-term reduction of TPB populations in the region and is not seen as a short-term solution to TPB control.

CONCLUSIONS.

Grower participation is essential to successful implementation of biological control, particularly in high-value crops such as strawberry. A team approach enabled development of a set of tools, Pre-growing season – Grower Workshops; During growing season – On farm discussions; Post-season – Grower report template; and Post-project – Final questionnaire, that increased grower participation. Awareness of the TPB life cycle and biological control options were greatly increased by growers who used this farmer participatory approach. A participatory approach like the one presented here, will also help to create a more positive perception by farmers towards biological control as a useful part of IPM programs.

ACKNOWLEDGEMENTS

We would like to thank the Pest Management Centre, Ottawa for research funding; the growers for their field sites and enthusiasm; our lab technicians for supervising the summer students and in London directing the production of *Lygus* and *Peristenus* [London: Lola Gualtieri, Lou Verdon, Richard Muth; Ottawa: Andrea Brauner]; and the summer students 2006-08 [Southern Ontario: Meghan Pelletier lead 2006, Karen Choi lead 2007, James Knechtel lead 2008, Mandy MacGillivray, Lauren Block, Candace Cole, Divid Hawil, Christie Martin and Kevin Macwan; Eastern Ontario: Jake Miall, Kathy Makela, Tom Hay, Melaika Soule] who competently visited the farms and collected the data.

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Poster abstracts

SESSION 1

NEW AND EMERGING SUCCESSES IN CLASSICAL BIOLOGICAL CONTROL: HAS THEORY IMPROVED PRACTICE??

DEVELOPING HOST RANGE TESTING FOR *COTESIA URABAE*: THEORY INFORMING PRACTICE

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Classical biological control is currently under development for *Uraba lugens* (Lepidoptera: Noctuidae), an Australian *Eucalyptus* (Myrtaceae) pest recently established in New Zealand. The solitary larval endoparasitoid *Cotesia urabae* Austin & Allen (Hymenoptera: Braconidae) is the agent currently under investigation. Host range testing procedures have been developed according to recent published theory, taking into account the biology of the insects and resources available. A non-target species list was compiled based on relatedness to the target host. Small arena sequential no-choice tests were used to determine physiological host preferences of the parasitoid for these species. This was followed by choice and no-choice tests in larger arenas to examine host finding abilities. Initial results have shown that this parasitoid will attempt to attack a number of non-target species, one of which may be a marginal physiological host. Further research is planned to expand our understanding of host finding and potential host range at an ecological scale to determine what level of risk the laboratory results represent.

EFFECTS OF A FOOD AND WATER-FEEDING DEVICE FOR MASSIVE REARING OF *MALLADA BASALIS* (WALKER) (NEUROPTERA: CHRYSOPIDAE)

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The green lacewing, *Mallada basalis* (Walker) (Neuroptera: Chrysopidae) is a predatory insect of agricultural pests commonly found on many crops in Taiwan. Four kinds of pollen, e.g. roxburgh sumac pollen, tea pollen, rape pollen and water lily pollen were studied. The results indicated that the roxburgh sumac pollen was the best food source for an increase in the longevity and fecundity of the green lacewing, it revealed that the food protein was the major factor for the nutrition of the green

lacewing. The results may be used in support of improvements in mass production and release programs of the lacewing. A new type of handmade water-feeding device made of a waste film case with a cover and a strip of gauze was used in place of the conventional water-supplying feeder for mass rearing trials of the adult green lacewing, *M. basalis*. The results indicated that the new type water-feeding device showed improved efficiency, lowered the cost, and prolonged the longevity of this insect in comparison with the conventional water-supplying feeder for the mass rearing of adult green lacewings. The overall performance of the cost-saving new-type water-feeding device for mass production of the adult green lacewing for field releases in bio-control programs is described.

FACTORS CONTRIBUTING TO THE EMERGING SUCCESS OF IRISH *MICROCTONUS AETHIOPOIDES* AS A BIOLOGICAL CONTROL AGENT FOR *SITONA LEPIDUS* IN NEW ZEALAND

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In 2006, a parthenogenetic strain of the braconid endoparasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) was released for the control of the clover root weevil *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae) in New Zealand pastures. Establishment was rapid with parasitism exceeding 85% at all four initial release sites before and during winter 2008. Host population suppression is evident and natural dispersal is taking place, with parasitism detected over 15 km into surrounding farmland in just over two years. The factors contributing to the success include: (1) search and screening processes evolved from knowledge gained in previous introductions of *Microctonus* spp. for control of New Zealand weevil pest species; (2) robust risk assessment; (3) attributes of the relatively biologically simple New Zealand pasture ecosystem and (4) the biology and phenology of Irish *M. aethiopoides*, in particular parthenogenesis and host: parasitoid synchrony.

THE POTENTIAL FOR CLASSICAL BIOLOGICAL CONTROL OF *DELIA RADICUM* BY *ALEOCHARA BIPUSTULATA*

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Delia radicum (L.) (Diptera: Anthomyiidae) is an increasingly important pest of canola in western Canada, and is of Palaearctic origin. The parasitoid community of *D. radicum* in canola in Canada and Europe is dominated by *Aleochara bilineata* (Gyllenhal) (Coleoptera: Staphylinidae) and *Trybliographa rapae* (Westwood) (Hymenoptera: Figitidae). In addition, in Europe, *Aleochara bipustulata* L. (Coleoptera: Staphylinidae) parasitizes *D. radicum*, particularly in spring-seeded canola. Studies of interactions between the *Aleochara* spp. suggest that the addition of *A. bipustulata* to the Canadian community will not disrupt parasitism by *A. bilineata* and may increase total *Aleochara* parasitism. Host selection involves female *Aleochara* selecting oviposition sites near *D. radicum* puparia, and the newly-hatched *Aleochara* larva successfully entering a puparium and parasitizing the pupa. No-choice laboratory assessments of *A. bipustulata* larvae indicate that close relatives of *D. radicum* and some less closely-related Diptera with small puparia are suitable hosts. However, assessment of non-target risk requires an understanding of oviposition site selection, and at least some hosts that are suitable for *A. bipustulata* larvae are not at risk due to ecological separation. Results of recent studies on host and prey selection and interactions among natural enemies are presented.

CLASSICAL BIOLOGICAL CONTROL OF EXOTIC SPIRALLING WHITEFLY *ALEURODICUS DISPERSUS* RUSSELL IN INDIA

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The spiralling whitefly *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), native to Caribbean islands and Central America, was first reported in India in 1993 in Western Ghats of Kerala and Kanyakumari district of Tamil Nadu. Later it spread to other states such as Karnataka, Andhra Pradesh, Maharashtra, the Lakshadweep islands, etc. The spiralling whitefly is known to attack over 500 plant species including fruits, Vegetables, ornamentals, etc. Chemical control was not possible as the nymphs are covered with heavy waxy flocculent materials and it was also impracticable to use on avenue trees in urban areas and inaccessible areas like forests. Application of insecticides temporarily only reduced the whitefly abundance. The local natural enemy complex in India failed to suppress *A. dispersus*. Therefore, alternatively classical biological control method was envisaged. The aphelinid parasitoids *Encarsia* sp., *E. (?) haitiensis* Dozier *E. guadeloupae* Viggiani (Hymenoptera: Aphelinidae) were known to cause high parasitism and provided excellent control of *A. dispersus* in countries where these parasitoids were accidentally or deliberately introduced. In India, accidentally introduced *Encarsia* sp., *E. (?) haitiensis* and *E. guadeloupae* were mass reared and inoculative introductions were made in several parts of the country since 1999 for permanent establishment and control of spiralling whitefly. The survey in South India during 2001-2002 indicated that *E. guadeloupae* had established and was dominant over *E. (?) haitiensis*. The paper also discusses successful control of spiralling whitefly on

guava, banana, ornamental plants, etc. and updates the status of classical biological control attempts in India.

ESTABLISHMENT AND IMPACT OF EXOTIC APHELINID PARASITOIDS ON *BEMISIA TABACI*: A LIFE TABLE APPROACH

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A large multi-institutional, interagency classical biological control program was initiated in the early 1990s to combat the invasion of the B biotype of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) into the USA. This large program was successful in the discovery, importation, rearing and release of more than 30 species/strains of aphelinid parasitoids (primarily *Eretmocerus* and *Encarsia*) from around the world into multiple states. Establishment of several species in each targeted state has been documented. In Arizona, two exotic species, *Eretmocerus* nr. *emiratus* Zolnerowitch & Rose and *Encarsia sophia* (Girault & Dodd) (Hymenoptera: Aphelinidae), have become established and have largely displaced native aphelinid species attacking *B. tabaci*. However, the impact of these establishments and the overall biological control program in Arizona and elsewhere has been poorly documented. From 1996 through the present, *in situ* life tables have been constructed for *B. tabaci* on cotton in central Arizona. Analyses of these life tables demonstrate that parasitism varied across years at low to moderate levels but that there is no trend for increasing levels of parasitism since the exotics became established. Additional analyses showed that the irreplaceable mortality supplied by parasitism has not consistently increased since establishment and that parasitism has no explanatory value in predicting total generational mortality. Predation by sucking predators has consistently been the largest source of mortality, has consistently contributed the largest amount of irreplaceable mortality and represents the key factor explaining variations in total mortality both before and after the establishment of exotic aphelinids.

ENTOMOPATHOGENIC FUNGI MAY COLONISE LEAVES OF COTTON

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Endophytes inhabit plant tissues for at least part of their life cycle without harming their host. The fungi *Lecanicillium lecanii* (Zimmerman) Zare & W. Gams (Hyphomycetes), *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes) and *Metarrhizium anisopliae* (Metschnikoff) Sorokin (Hyphomycetes) are important entomopathogens in the glass house and field, though their life cycle remains

unclear. An isolate of *L. lecanii* from leaves of cotton, and *B. bassiana* and *M. anisopliae* from dead insects were investigated to determine whether the entomopathogenic fungi can readily colonise leaves of cotton. Fully expanded leaves of cotton grown in growth chambers were sprayed with spore suspensions of the various fungi. After one to 21 days leaves from inoculated and uninoculated plants were removed, surface sterilized, cut into one cm segments and plated on PDA. Overall, colonization was highly variable, and though colonization declined over time, at least some leaves remained colonized for up to 3 weeks. As age of leaf may influence the rate colonization, a subsequent experiment with *L. lecanii* examined the relative potential for the most recently expanded and the next two oldest leaves to become colonized. The fully expanded leaf was more heavily colonized than the younger leaves. While aspects of the life cycle remain to be confirmed, these data support the view that cotton plants may become colonized by entomopathogenic fungi, and this stage may be used in biocontrol of insect pests of cotton.

BIOLOGICAL CONTROL POTENTIAL OF *USCANA FEMORALIS*, AN EGG ENDOPARASITOID OF *CALLOSOBRUCHUS THEOBROMAE* (BRUCHIDAE: COLEOPTERA)

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Uscana femoralis Pajni & Sood (Hymenoptera: Trichogrammatidae), recorded for the first time from the eggs of *Conicobruchus albopubens* (Pic) (Coleoptera: Bruchidae), was found parasitizing the eggs of *Callosobruchus theobromae* (L.) (Coleoptera: Bruchidae), a serious pest of different legume commodities in India. The parasitoid deposited its eggs inside the eggs of host insect by puncturing through the egg chorion with its ovipositor. Host eggs 0-84 h old were accepted for parasitization. Parasitoid eggs hatched inside the host eggs and further development was completed inside the host egg consuming the host egg ooplasm. Adult parasitoids eclosed after 9.4 ± 0.46 days by making a circular cut from inside the eggshell of the host. Of the total eggs of the host insect deposited on seeds of its host plant, 22% were destroyed by the parasitoid thus resulting in biocontrol of host insect.

NON-CROP VEGETATION AND COLONIZATION OF *PERISTENUS RELICTUS* FOR CONTROL OF LYGUS, A PEST OF STRAWBERRIES

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Lygus bugs are a key pest of strawberries grown in the Monterey Bay region along the central coast of California. Although native to North America, multiple surveys have found they lack nymphal parasitoids. Two species of braconids associated with European species of Lygus were introduced into non-crop vegetation for their intentional, permanent establishment. Beginning in 2002, parasitoids were released into wild vegetation surrounding or near conventionally-managed fields of strawberries. They were next released into Lygus specific strips of alfalfa, *Medicago sativa* L. (Fabaceae) interplanted as a trap crop in organically produced strawberries. One of the two species, *Peristenus relictus* Loan (Hymenoptera: Braconidae) has been found attacking Lygus bugs in strawberries five years after last being released into nearby wild vegetation. The same parasitoid has been found attacking Lygus bugs in organically produced strawberries three years after being released into interplanted strips of alfalfa. Populations of *P. relictus* were significantly correlated with Lygus collected from alfalfa trap crops from 2005 to 2007 ($r^2 = 0.60$; $p < 0.005$). Lygus populations at this site have dropped from a high of 2.7 nymphs per 50 suctions at the beginning of this study to 0.8 nymphs per 50 suctions in 2007.

SUPER PARASITISM BY *PLATYGASTER DEMADES*, AN EGG PARASITOID OF APPLE LEAF CURLING MIDGE

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Super parasitism by *Platygaster demades* Walker (Hymenoptera: Platygasteridae) occurs when the parasitoid population is high and the apple leaf curling midge (ALCM), *Dasineura mali* Kieffer (Diptera: Cecidomyiidae), population is low at the end of summer. A study was conducted to help understand the impact of super parasitism on the parasitoid and pest populations. The numbers of *P. demades* pupae in each parasitised ALCM larva were recorded from a sample of 500 mature ALCM larvae extracted from infested apple leaves collected from the field. Fifty nine percent of the ALCM larvae were parasitised and 1-5 *P. demades* pupae developed in a single ALCM larva. A single *P. demades* pupa was found in 42.9% of those parasitised ALCM larvae and 2, 3, 4 or 5 pupae were found in 38.8%, 16%, 1.7% and 0.7% of parasitised larvae, respectively. Adult *P. demades* emerging from single pupae were significantly larger in size than those emerging from super parasitised ALCM. Parasitoids emerged from multiple pupae within a single ALCM larva at different times, within a period of 1-3 days. The multiple parasitoid numbers emerging from super parasitised ALCM larvae may have an impact on both pest and parasitoid populations through competition for resources.

REPRODUCTIVE STRATIGY AND BIOLOGICAL CONTROL
POTENTIAL OF *EURYTOMA RAOI*, A KOINOBIONT PARASITOID OF
CARYEDON CRINEUS (COLEOPTERA: BRUCHIDAE)

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In recent years, increased attention towards non-chemical methods of stored product protection has been made because chemical control is often not affordable for subsistence farming, whereas the available chemicals pose a serious health risk due to lack of appropriate facilities, and knowledge and lack of training. In the present study it was observed that the eurytomid, *Eurytoma raoi* Narendran (Hymenoptera: Eurytomidae) parasitized the larva of *Caryedon crineus* Arora (Coleoptera: Bruchidae). Gravid females of *E. raoi* laid eggs directly on the body of the last instar larva of *C. crineus*. Although larval development of *C. crineus* was completed inside the host seed within the pod, the last instar larva emerges from the infested seed in search of a suitable place for cocoon formation and becomes the victim of a gravid parasitic wasp.

SESSION 2

BIOLOGICAL CONTROL AND ENVIRONMENTAL/CLIMATE CHANGE

EFFECT OF FOUR LARVICIDAL BOTANICAL BARK EXTRACTS FROM *ACACIA AURICULIFORMIS* (A. CUNN.) AGAINST MELON FRUIT FLY, *BACTROCERA CUCURBITAE* (COQUILLET)

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The rapid deterioration in the Environment and Public health is an area of major concern, which has been partially attributed to indiscriminate and excessive use of pesticides. There is a need to replace conventional pesticides with some alternative safe and ecofriendly compounds. In the last few years, biopesticides, derived from biological organisms are gaining considerable attention as natural compounds for use in pest control. *Acacia auriculiformis* A. Cunningham ex. Benth. (Fabaceae) is an important medicinal plant and widely distributed member of the legume family. It has been reported to be a rich source of polyphenols along with terpenoids (Wallings 2000; Singh & Sehgal 2001). In the present study the influence of four extracts from the bark of *A. auriculiformis* (Methanol, Acetone, Ethyl acetate and Water extract) were investigated on the growth and development of the second instar larvae of melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), a major pest of crops of the cucurbitae family. The larvae of *B. cucurbitae* were fed on artificial diet incorporated with different concentrations (1, 5, 25, 125, 625 ppm and control) of the four bark extracts of *A. auriculiformis*. The larval and total development periods were prolonged after treatment of the larvae with all four extracts. The pupation and emergence were significantly reduced. The most notable effect was observed in the treatment of the larvae with ethyl acetate extract as revealed by LC50 value of 177.8 ppm compared to the LC50 value of 316.2 ppm for the methanol extract, 251.2 ppm for the acetone extract, and 354.8 ppm for the water extract.

ISOLATION AND IDENTIFICATION OF A BACTERIUM FOR DEGRADING HIGH CONCENTRATION NICOTINE

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One hundred and fifty-two bacterial strains which were able to utilize nicotine as their sole carbon and nitrogen sources were isolated from soils of tobacco plantings

in Zi Bo, Shan Dong and Waste in Wuhan Tobacco Company, respectively. Strain ZB-16A can degrade high concentrations of nicotine. By morphological observation, physiological and structural characteristics, and by the comparison of its 16S rDNA analysis, the strain ZB-16A was identified as *Pseudomonas putida* (Trevisan) Migula (Pseudomonadaceae). Strain ZB-16A could tolerate 6000 mg/L nicotine and all the nicotine was degraded after 48hr when the temperature, initial pH and rotation speed is 30°C, pH 7.0 and 150 rpm, respectively. ZB-16A growth was inhibited in 7000 mg/L nicotine concentration. This research could provide scientific basis for bioremediation of nicotine pollution.

INTRINSIC COMPETITION BETWEEN TWO OLIGOPHAGOUS PARASITIDS, *STURMIOPSIS PARASITICA* AND *COTESIA SESAMIAE*, ATTACKING THE SAME LIFE STAGES OF LEPIDOPTERAN CEREAL STEMBORERS

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Host acceptability and suitability of four cereal stemborers commonly occurring in eastern Africa, *Sesamia calamistis* Hampson and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), and *Eldana saccharina* Walker (Lepidoptera: Pyralidae), to a West African strain of *Sturmiopsis parasitica* (Curran) (Diptera: Tachinidae) were assessed. In addition, the outcome of multiparasitism was studied using a local strain of the endoparasitic *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) as the competing parasitoid. Various parasitism sequences and time intervals between parasitism were chosen. Parasitism increased linearly with the number of planidia used per larvae and was 80% with eight planidia. All species were accepted for larviposition but suitability varied greatly; parasitism was 75.2, 37.9, 34.8, and 23.8% by *S. calamistis*, *B. fusca*, *E. saccharina*, and *C. partellus*, respectively. *Sturmiopsis parasitica* outcompeted *C. sesamiae* irrespective of the time interval between parasitism, and whether it was the first or second species to parasitize. This was mainly due to a longer egg-to-cocoon development time and a high cocoon-to-adult mortality in *C. sesamiae*. The implications of these results for expanding the geographic range of the West African strain of the tachinid in Africa are discussed.

FERTILITY LIFE TABLE AS A FACTITIOUS HOST SELECTION CRITERION FOR THREE TRICHOGRAMMATIDAE SPECIES.

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We aimed to select the most suitable factitious host for three trichogrammatids, *Trichogramma atopovirilia* Oatman & Platner, *T. bruni* Nagaraja and *Trichogrammatoidea annulata* Se Santis (Hymenoptera: Trichogrammatidae), by using fertility life tables. The mean generation time (T), net reproductive rate (R_0), intrinsic rate of natural increase (r_m) and the finite rate of increase (r) were estimated in each one of the three factitious hosts tested, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae), and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). *Corcyra cephalonica* was the most suitable host for rearing *T. bruni* and *T. annulata*, while for *T. atopovirilia*, *C. cephalonica* was as suitable as *A. kuehniella*. On the other hand, *S. cerealella* was found to be an inadequate host for all trichogrammatids evaluated, as the parasitoid population capacity of increase was dramatically reduced on all hosts. Therefore, the use of fertility life tables can aid on the host selection process for trichogrammatid mass rearing.

BUGS (INSECTA: HETEROPTERA) FROM RICE FIELDS AND SURROUNDING GRASSLANDS OF NORTHERN IRAN, WITH SPECIAL STUDY ON PREDATOR SPECIES

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The predatory bugs (Heteroptera) have an important role in biological control of several pests in different agro-ecosystems. The diversity of these beneficial insects was studied in rice fields and around grasslands of North and North Western Iran, Mazandaran, Guilan, Golestan and East Azarbayjan provinces during 2002-2006. The specimens were collected mainly by sweep net and aspirator from the rice fields and around grasslands. All the specimens were determined by the third author. In total, 37 Heteroptera species were collected of which 12 species including, *Rhinocoris iracundus* (Poda), *Rhinocoris monticola* (Oshanin), *Reduvius dorsalis* Stal, *Sinea diadema* (Fabricius), *Zelus biblobus* (Say) (Hemiptera: Reduviidae), *Anonychiella brevicornis* (Reuter), *Grypocoris ajderensis* Putshkov, *Orthotylus achilleae* Putshkov, *Phytocoris thragacanthae* Putshkov, *Pilophorus disjunctus* Kerzhner, *Psallus (Phyllidea) dichrous* Kerzhner (Hemiptera: Miridae), and *Gerris lacustris lacustris* (L.) (Hemiptera: Gerridae) are new records for Iran. To preserve the diverse heteropteran predator fauna in Iranian rice fields, pesticides must be used only as necessary and especially in IPM and ICM programs where the conservation of natural enemies is a priority.

EFFECT OF ANTIBIOTIC ON THE NATURAL ENEMY, *NEOCHRYSOCHARIS FORMOSA*

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Many natural enemies are commercially used worldwide. The use of these natural enemies has many advantages. Fecundity is one of the important ecological characteristics of a natural enemy. Recently, many types of intracellular symbionts that affect fecundity of the host parasitoid, such as *Wolbachia* (Anaplasmataceae) and *Rickettsia* (Rickettsiaceae), have been reported in insects. *Wolbachia* and other intracellular symbionts induce many kinds of reproductive alterations in host insects, such as cytoplasmic incompatibility, feminization of genetic males, male killing, and parthenogenesis induction. When the symbiont induces parthenogenesis against host parasitoids, the infected females produce only uninfected males by antibiotic treatment. These males are not effective as natural enemies of pest insects. By using antibiotics, insect intracellular symbionts can easily be removed. Antibiotics are naturally used to treat plant diseases. We tested the effect of an antibiotic against the leaf-miner parasitoid, *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) that was infected with *Rickettsia*. *Rickettsia* induced parthenogenetic (thelytokous) reproduction of the host *N. formosa*. We treated the primary leaves of a kidney bean containing third instar larvae of *Liriomyza trifolii* (Burgess) (Diptera: Liriomyzidae) with oxytetracycline. The results revealed that *N. formosa* produced only male offspring. *Neochrysocharis formosa* ingests the antibiotic not only by feeding on an antibiotic containing host *L. trifolii*, but also thorough mouthpart contact to the surface of treated leaves. These results indicate that antibiotics may decrease the effect of natural enemies and the sustainable utilization of the natural enemy.

CO-ADAPTATION OF AN ENTOMOPATHOGEN WITH THE POMEGRANATE APHID, *APHIS PUNICAE* PASSERINI

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Pomegranate, *Punica granatum* Linn. (Punicaceae) is an important source of antioxidants. The aphid, *Aphis punicae* Passerini (Hemipera: Aphidae) is a pest on pomegranate. An entomopathogenic fungus (EPF), *Entomophthora aphidis* Hoffman (Zygomycetes: Entomophthorales) causes high mortality of the aphid. Therefore, it was felt that understanding the relationships among the weather, crop phenology, aphid and EPF would be useful in aphid management. From 2002-2004, a study was conducted in a pesticide-free orchard in Bangalore, (12°58'N, 77°35'E) India. Weekly samplings were done on 20 random trees (out of 80) on 80 random shoots per tree. Thus, 1600 shoots/week were sampled for aphids and graded for pathogen infection. These data were subjected to correlation matrix analysis with the weather and crop phenology factors. It was found that the infection of the pathogen on the

aphids showed a positive correlation with tender and half matured leaves and negative correlation with flowering and fruiting. The EPF also showed a negative correlation with relative humidity and minimum temperature, which was useful, as even at lower relative humidity *E. aphidis* was able to infect *A. punicae*. Interestingly, in aphids also, the correlation trends with the above phenological and weather factors were similar to the EPF's. We infer that this is evidence of the EPF co-adapting with the aphid and its environmental variables (=niche) and hence explains its high efficacy. We speculate that such co-adapted EPF strains may also show regional eco-specificity. If mass produced it will be a useful and environment-friendly input in the IPM of the aphid.

SESSION 3

EXPLORING BIOLOGICAL CONTROL TO MANAGE NEW OR POTENTIAL INVASIVE ALIEN PESTS

BIOLOGY AND FEEDING EFFICIENCY OF INDIGENOUS PREDATORS OF COTTON MEALYBUG, *PHENACOCCLUS* *SOLENOPTIS* TINSLEY (HOMOPTERA: PSEUDOCOCCIDAE)

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Cotton mealybug has become a serious pest for the last few years in the cotton belt of Punjab, Pakistan. The role of beneficial insects, especially predatory insects can help to limit its spread from alternate host plants to major economic crops like cotton, etc. The biologies of four predatory coccinellid beetles, *Brumoides suturalis* (Fab.), *Coccinella septempunctata* L., *Menochilus sexmaculatus* (Fab.), and *Scymnus nubilus* Mulsant (Coleoptera: Coccinellidae), and *Chrysoperla carnea* (Stevens) (Neuroptera: Chrysopidae) were studied under controlled laboratory conditions. *Menochilus sexmaculatus* and *B. suturalis* showed the shortest mean life duration (~20 days) followed by *S. nubilus* (22.2 days), *C. septempunctata* (25.6 days). *C. carnea*, on the other hand, took the longest to complete its life cycle (28.3 days). Larval development ranged from 12.2 to 15.3 days, with the highest feeding efficiency by *C. septempunctata* (153 mealybug nymphs consumed) amongst coccinellid beetles. *Chrysoperla carnea* larvae proved to be efficient feeders of the first instar nymphs of mealybug consuming 14.1 per day (216 in total). Incorporation of *C. carnea* with indigenous coccinellid beetles having both larval and adult predatory stages can help in better management of cotton mealybug, a noxious insect pest of more than 150 host plants.

ENTOMOPHAGOUS FUNGI ASSOCIATED WITH INSECT PESTS IN CASHEW ORCHARDS OF TAMIL NADU, INDIA

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A 10 year field survey of cashew orchards in Tamil Nadu (India) from 1998-2007 identified 12 genera of entomophagous fungi from 22 species of insects representing six orders. The fungal genera identified in order of occurrence include seven Hyphomycetes: *Beauveria*, *Metarhizium*, *Paecilomyces*, *Nomuraea*, *Verticillium*, *Aspergillus* and *Fusarium*, and five Zygomycetes: *Conidiobolus*, *Entomophthora*, *Pandora*, *Neozygites*, *Mucor* and *Rhizopus*. Of 46 species of cashew insects prevalent, 22 were positively infected with one or more species of fungi, with 16.6-43.3% mycoses under natural conditions. The cashew stem and root

borer, *Plocaederus ferrugineus* Linn. (Coleoptera: Cerambycidae), the termite, *Odontotermes obesus* (Rambur) (Isoptera: Termitidae), leaf folder, *Sylepta aurantiacalis* Fisch (Lepidoptera: Pyralidae), mirid bug, *Helopeltis antonii* Signoret (Hemiptera: Miridae), aphid, *Toxoptera odinae* van der Hoot (Hemiptera: Aphidae) and flower thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) were used for assessing pathogenicity of fungi. Among the fungi, *Beauveria bassiana* (Bals.) Vuill. was highly pathogenic to all the test insects causing 76.6-100% mortality under laboratory conditions. While, *Metarhizium anisopliae* (Metsch) Sorokin, *Paecilomyces farinosus* (Holm ex S F Grey) Brown and Smith and *Verticillium lecanii* (Zimmerman) Veigas inflicted 53.3-96.6% mortality to one or more species of Coleoptera, Lepidoptera, Hemiptera and Isoptera. However, the five fungal Zygomycetes genera were host specific mainly confined to thrips/aphids/mealy bugs and caused mortality below 30.0% under pathogenicity tests. The Zygomycetous *Conidiobolus obscures* (Hall & Dunn) Remaudiere & Kellerr, *Neozygites fresenii* (Nowakowski) Remaudiere & Kellerr and *Pandora neoaphidis* (Remaudiere & Hennebert) Humber have not previously been reported from cashew insects.

RELATIVE VIRULENCE OF ENTOMOPATHOGENIC HYPHOMYCETES FOR BIOLOGICAL CONTROL OF CASHEW MIRID BUG, *HELOPELTIS ANTONII* SIGNORET (HETEROPTERA: MIRIDAE)

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Twenty isolates of entomopathogenic fungi (Hyphomycetes) including 10 *Beauveria bassiana* (Bals.) Vuill., six *Metarhizium anisopliae* (Metsch) Sorokin and four *Paecilomyces farinosus* (Holm ex S.F. Grey) Brown & Smith derived from a range of insect species were evaluated *in vitro* against the mirid bug, *Helopeltis antonii* Signoret (Heteroptera: Miridae), a serious sucking pest of cashew. The bioassay had two major components: the initial single dose (1×10^7 conidia/ml) time mortality assays followed by multiple-dose (1×10^5 to 1×10^9 conidia/ml) assays each replicated four times. Conidial suspensions of respective isolates prepared in 0.05% Tween 80[®] were sprayed topically after release of 20 third instar nymphs per replication in mylar-film cages each containing four cashew grafts previously dipped in the respective fungal suspension. Assessment of infection was made every 24 h from 4 to 12 days post treatment. All the isolates tested were capable of infecting *H. antonii*, but their virulence determined by LT₅₀ and LC₅₀ varied. In single-dose (1×10^7 conidia/ml) screening assay, five isolates including three *B. bassiana*: BbHa VRI 0102, BbFv VRI 0201 and BbSI TIR 1201; and two *M. anisopliae*: MaFv VRI 0202 and MaPf VRI 0198 isolates were superior to all other isolates with mirid bug derived *B. bassiana*: BbHa VRI 0102 inflicted quickest LT₅₀ in 5.9 days. In multiple-dose assays, the mirid bug- derived *B. bassiana* isolate, BbHa VRI 0102 inflicted the greatest mortality at a concentration lower than the standard dose of 1×10^7 conidia/ml. Based on the time-

dose pathogenicity, the *B. bassiana* isolate, *BbHa* VRI 0102, has been selected for further field studies.

INTEGRATION OF THE PARASITOID *DIAERETIELLA RAPAE* (MCINTOSH) (HYMENOPTERA: APHIDIIDAE) AND CERTAIN INSECTICIDES TO CONTROL THE CABBAGE APHID *BREVICORYNE BRASSICAE* L. (HOMOPTERA: APHIDIDAE)

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This study was an attempt to search for insecticides that are of high degree of toxicity to the cabbage aphid, *Brevicoryne brassicae* L. (Homiptera: Aphidae) and of low toxicity to its aphidiine parasitoid wasp, *Diaeretiella rapae* McIntosh (Hymenoptera: Braconidae), as part of an integrated pest management program. Six insecticides that are used in Jordan for cruciferous crop protection were evaluated. These insecticides are: Actara® (thiamethoxam, Neonicotinoide), Calypso® (thiacloprid, Neonicotinoide), Actellic® (pirimiphos-methyl, Organophosphate), Malathion® (malathion, Organophosphate), Pirimor® (pirimicarb, Carbamate), and Cypermethrin® (cypermethrin, Pyrethroide). There were large differences between the LC₉₀s of the insecticides on the cabbage aphid and the recommended field rates of these insecticides. From the ratios of LC₅₀s estimated for the aphid to those estimated for the parasitoid, the two insecticides Cypermethrin® and Pirimor® gave a ratio of less than one which means that they are more toxic to the aphid than to its parasitoid. Thus, they are good candidates to be used in integrated pest management programs (IPM) for the cabbage aphid.

NATURAL ENEMIES OF *TOXOPTERA CITRICIDA* (KIRKALDY) (HEMIPTERA: APHIDIDAE) AND OTHER CITRUS APHIDS IN SPAIN

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The aphid *Toxoptera citricida* (Kirkaldy) (Homiptera: Aphidae), the most efficient vector of *Citrus tristeza virus* (CTV) (Closteroviridae) in the world, is present on citrus along the Atlantic coast in the northwest quadrant of the Iberian Peninsula, but it is absent from the main Spanish citrus areas in eastern and southern Spain. *Toxoptera citricida* is attacked in northern Spain by 18 species of natural enemies, most of them present in eastern Spain on other citrus aphids. In northern Spain, the main natural enemies of *T. citricida* are Syrphidae, Coccinellidae and Aphidiinae, and CTV spread

has not been detected. In eastern Spain, the main natural enemies of citrus aphids, *Aphis spiraecola* Patch and *A. gossypii* Glover (Hemiptera: Aphidae), are Cecidomyiidae and Coccinellidae.

STUDY OF THE PARASITIDS OF *LIRIOMYZA TRIFOLII* (DIPTERA: AGROMYZIDAE) IN SHIRAZ, IRAN

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A survey was conducted to identify parasitoids of vegetable leafminer *Liriomyza trifolii* (Burgess, 1880) (Diptera: Agromyzidae) in Shiraz region during April 2004 – September 2005. A total of 13 eulophid species (Hymenoptera: Eulophidae) were recognized. Four species which are marked with a (*), are new records from Iran.

1. *Baryscapus impeditus* Nees*
2. *Baryscapus* sp.
3. *Closterocerus formosus* Westwood
4. *Closterocerus* sp.
5. *Diaulinopsis arenaria* Erdos*
6. *Chrysocharis* sp.
7. *Diglyphus crassinervis* Erdos
8. *Diglyphus isaea* Walker
9. *Diglyphus pulchripes* Crawford*
10. *Hemiptarsenus zilahisebessi* Erdos
11. *Ratzeburgiola cristatus* Ratzeburg*
12. *Pnigalio* sp.
13. *Pediobius* sp.

The Bulgarian specialist, Dr. Boyadzhiv, confirmed the parasitoids identifications. All of the specimens were deposited in the insect collection of the Jahrom Azad University.

AN APPROACH TO BIOLOGICAL CONTROL OF *ZOPHIUMA LOBULATA* GHAURI (HOMOPTERA: LOPHOPIDAE) USING MYMARID AND ENCYRTID EGG PARASITIDS

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The plant hopper *Zophiuma lobulata* Ghauri (Hemiptera: Lophopidae) has been implicated to Finschhafen disorder of coconut and oil palms in Papua New Guinea. The biology and the pest status of *Z. lobulata* are largely unknown. Previous reports suggest that the disorder is prevalent when and where the plant hopper is present and is continuously feeding on the palms. The control of the disorder is therefore dependent on the management of *Z. lobulata* populations. Egg masses of *Z. lobulata* collected from coconut and oil palms in two seasons during 2007 and 2008 yielded parasitoids belonging to the families Mymaridae and Encyrtidae. This poster will draw attention to the importance of these egg parasitoids in the control of *Z. lobulata* in Papua New Guinea.

BIOPESTICIDES: AN IDEAL COMPONENT OF IPM IN CHILI UNDER IRRIGATED ECOSYSTEM

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Chili, *Capsicum annuum* L. (Solanaceae), commonly known as red pepper is an important and indispensable condiment as well as vegetable grown in many parts of the world. To combat the insect and mite pest outbreaks, farmers are solely dependent on pesticides. In Northern Karnataka, farmers apply nearly 18-26 rounds of chemical sprays of different combinations of pesticides under irrigated ecosystems (Anon. 2006). Three modules viz., Biointensive module, Adoptable module and Farmers practice were tested during 2004-2005 and 2005-2006 kharif season. The Biointensive module included neem-based insecticides and biopesticides like HaNPV and SINPV as major components, the Adoptable module used the combination of effective new molecules with biopesticides, and the Farmers practice was based solely on chemical insecticides. The pooled data indicated that the Adoptable module recorded fewest thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), mites, *Polyphagotarsonemus latus* Banks (Acari: Tarsonemidae), and fruit borer, *Helicoverpa armigera* Hub. (Lepidoptera: Noctuidae), populations, followed by the Farmers practice and the Biointensive module. Use of HaNPV and SINPV was found to be an effective component in the Adoptable and Biointensive modules which recorded the minimum larval populations. The Adoptable module recorded maximum dry chili yield of 16.08q/ha which was superior to the Farmers practice (15.00q/ha) and the Biointensive module (11.12q/ha). The Biointensive module recorded maximum predator populations and it was on par with the Adoptable module while the Farmers practice recorded very low predator populations.

PROSPECTS FOR THE BIOLOGICAL CONTROL OF THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* (COLEOPTERA: CURCULIONIDAE), IN THE MEDITERRANEAN

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Rhynchophorus ferrugineus (Olivier) (Coleoptera: Curculionidae) is an important pest of palms native to South East Asia. This species has recently colonized the Mediterranean Basin where it is a serious problem, especially on ornamental *Phoenix canariensis* hort. ex. Chabaud (Arecaceae) palms. This weevil is a concealed tissue borer which can spend all of its life stages inside the palm. Adults often remain within the host until the apical growing area of the palm has been destroyed, killing the palm. The methods currently used to control *R. ferrugineus* are chemical, with frequent applications over a long period of time. Nevertheless, efficacies are not high and there are deep concerns about the environmental impact of these treatments, especially in public areas where ornamental palms are grown. Investigations on the use of natural enemies of this weevil are underway. On the one hand, exotic parasitoids from *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae) from Ecuador have been identified and are currently under quarantine confinement, on the other, a native strain of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes) has been found in field-collected adults. This strain has shown a high virulence against all instars of the weevil in laboratory bioassays. Furthermore, a commercial formulation containing an indigenous strain of *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae) has shown efficacies around 80 % in curative semi-field assays, and up to 98 % in preventative assays.

A SURVEY OF NATURAL ENEMIES IN NEW ZEALAND CITRUS ORCHARDS INFESTED WITH *ORCHAMOPLATUS CITRI*

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Australian citrus whitefly (ACWF), *Orchamoplatus citri* (Takahashi), (Hemiptera: Aleyrodidae), was first detected in Auckland, New Zealand, in October 2000. It has since spread from Auckland to the major citrus growing regions of Kerikeri, Gisborne

and the Bay of Plenty, and south to at least Hawke's Bay. High populations are causing sooty mould problems and a possible reduction in fruit colour, as well as tree health issues. Conversely, in its native Australia, ACWF is only a minor, occasional pest on citrus, where it is assumed to be kept under control by its natural enemies. Two orchards in each of Kerikeri, Auckland and Gisborne areas with high populations of ACWF were selected and natural enemies on ACWF-infested leaf samples and yellow sticky traps were surveyed weekly or fortnightly from October 2007 to April 2008 to determine if effective natural enemies of ACWF were present. No parasitoids emerged from ACWF on leaf samples. Whitefly parasitoids generally belong to one of three hymenopterous families: Aphelinidae, Platygasteridae or Eulophidae. Of the 27 parasitoid species trapped, seven were in the Platygasteridae family and one in the Eulophidae family. Given the known host range of some of these species, and the absence of parasitized ACWF on leaf samples, none were likely to be attacking ACWF. Eight species of coccinellid ladybirds were trapped. No ladybird larvae or eggs were found on leaves and only adult *Halmus chalybeus* (Boisduval) (Coleoptera: Coccinellidae) were seen predating on ACWF, suggesting that ladybirds were having little impact on ACWF populations. Larvae of the lacewing *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) were also observed predating on ACWF and various species of predatory mites were associated with ACWF. Three species of Staphylinids were trapped, however none were seen consuming ACWF. None of the natural enemies collected in this survey appeared have any impact on the high ACWF populations observed in these orchards.

FEMALE *DIAERETIELLA RAPAE* RECEIVE MORE REPRODUCTIVE FITNESS GAIN BY PARASITISING OLDER *BREVICORYNE BRASSICAE* NYMPHS

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The reproductive fitness of parasitoids is closely associated with host quality. Understanding the oviposition strategy of parasitoids can help in improving their efficiency. To test the oviposition strategy and fitness of *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae), the females were given a choice of four different age groups (7, 5, 3 and 1 day old) of nymphs of the cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphidae). Ten hosts of each age group were offered daily to a mated and honey fed female until her death. The female appeared to show a stronger preference for the 7-day-old hosts for oviposition over younger hosts but was not able to discriminate between 1-day- and 3-day-old hosts. The development of female offspring was quicker in older hosts than in younger hosts. Males developed quicker than females in older hosts but their developmental time was not different in younger hosts. The proportion of females that emerged from older hosts was 73% which was significantly higher than those that emerged from younger ones. The body size of male and female offspring was positively correlated to the size of hosts in which they developed. Females that emerged from older hosts also lived for a significantly longer time, 4.6 ± 0.2 days, than those that emerged from younger ones. Results showed that the reproductive efficiency of *D. rapae* could be improved

by using older nymphs during mass production of the parasitoids in a biological control programme for *B. brassicae*.

THE ROLE OF *ANAGYRUS LOECKI* (HYMENOPTERA: ENCYRTIDAE) IN SUPPRESSING *PARACOCCLUS MARGINATUS* (HEMIPTERA: PSEUDOCOCCIDAE) IN COMMERCIAL PAPAYA FARMS IN HAWAII

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The papaya mealybug (PM), *Paracoccus marginatus* Williams and Granada de Willink (Hemiptera: Pseudococcidae), was first detected in Hawaii in 2004. This polyphagous species attacks many tropical crops and ornamentals. Current methods of control of PM in commercial papaya farms in Hawaii rely on the use of pesticides. The objectives of this study are to determine the density of PM in different papaya farms and to determine the effectiveness of the adventive parasitoid *Anagyrus loeckii* Noyes (Hymenoptera: Encyrtidae) in controlling PM in commercial papaya fields using emergence data. This paper will also compare parasitism rates of PM in commercial organic and non-organic papaya farms as well as present notes on the role of the adventive hyperparasitoid *Aprostocetus minutus* Howarth (Hymenoptera: Eulophidae) on the performance of *A. loeckii*.

EXPLORING USE OF IRRADIATED HOSTS FOR REARING FRUIT FLY PARASITIDS: A NOVEL APPROACH FOR INVASIVE TEPHRITID MANAGEMENT

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Fruit production in Australia is threatened by invasion of exotic fruit flies (Diptera: Tephritidae) as well as by a native species that has a limited distribution which is maintained by ongoing quarantine: the Queensland fruit fly ('Qfly'), *Bactrocera tryoni* (Froggatt). Biological control would be a welcome technology to respond to fruit fly incursions and help prevent spread of Qfly within Australia. This paper presents results from a study exploring the scope for mass rearing parasitoid wasps on gamma irradiated, sterile host material. The practical advantage of such a rearing system is that flies and wasps would not need to be separated, thus reducing cost of production. Host larvae of different stadia were irradiated at 0, 4.7, 9.1, 15.9,

27.6, 47.0 and 79.9 Gy and later exposed to *Diachasmimorpha kraussii* (Fullaway) (Hymenoptera: Braconidae) adults. Eggs were also irradiated (since these are likely to be easier to transport and handle) and exposed to parasitoids after eclosion and culturing on artificial medium. Detailed results will be presented for wasp development and subsequent reproductive performance as well equivalent data for the Qfly developing from unparasitised hosts. Overall results suggest good scope to develop a protocol involving a carefully calibrated irradiation dose and host material of a specific developmental stage that will allow mass production of *D. kraussii*, yet produces no flies from any unparasitised hosts. Findings will be discussed in relation to the wider potential for this approach in managing invasive tephritids.

LIFE TABLES OF *CACTOBLASTIS CACTORUM* (BERG) (LEPIDOPTERA: PYRALIDAE) IN CULTIVATIONS OF PRICKLY PEAR CACTUS (*OPUNTIA FICUS-INDICA*) IN ARGENTINA

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The cactus moth, *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) has become a serious threat to the diversity of native and cultivated *Opuntia* species in Mexico and the United States. These Cactaceae are important not only as crops, but also as factors in the maintenance of natural ecosystems. In August 2007, the South American Biological Control Laboratory (SABCL) started studies in Argentina to control this pest in North America. The moth mortality factors were identified by building life tables in managed and unmanaged cultivated fields of prickly pear cactus, *Opuntia ficus-indica* (L.) Mill. (Cactaceae) in Córdoba and Santiago del Estero provinces. In each field, more than 400 egg sticks were marked and monitored until the adult stage was reached in each of the 3 annual generations. Samples of the different stages were taken every 7-14 days. On average, the mortality of eggs, larvae, and pupae was 50%. Main mortality factors for eggs were rain and wind (11-29%) and ant predation (9-28%). For larvae and pupae, predation was the main mortality factor (46-50%). Parasitism was 2-6% for eggs (mainly by *Trichogramma pretiosum* Riley, and *Trichogramma* sp. (Hymenoptera: Trichogrammatidae)), 7-18% for larvae (mainly by *Apanteles alexanderi* Brethes (Hymenoptera: Braconidae)), and was negligible for pupae. In general, the sex ratio was 1:1. Although each generation showed an overall survivorship of only 1%, populations at both sites increased 6-7 fold from the initial values. Life table studies will be continued for one more year. Additionally, life table studies of *C. cactorum* will be carried out on native *Opuntia* at two new sites. Complementary studies on female moth oviposition preference and larval performance on native and exotic *Opuntia* species of Argentina are under appraisal.

INVESTIGATIONS INTO THE POTENTIAL BIOLOGICAL CONTROL OF THE INVASIVE SPECIES, *AGRILUS PLANIPENNIS* (COLEOPTERA: BUPRESTIDAE), IN NORTH AMERICA USING NATIVE PARASITIDS

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The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), was first discovered in North America in 2002 in the vicinity of Detroit, Michigan. The species is native to eastern Asia and dendrochronological evidence suggests it was present in the Detroit area for about 10 years prior to its discovery. USDA-FS evaluated the impact of native parasitoids on *A. planipennis* populations in 2003. Observations from Michigan populations indicated that native parasitism rates were <1%. We describe survey results that we have undertaken to assess parasitism in populations of endemic *Agrilus* spp. and in *A. planipennis*. We describe an Ontario population of *A. planipennis* that had high numbers of two larval parasitoids. The most abundant parasitoid was *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) and the less abundant parasitoid was *Balcha indica* (Mani & Kaul) (Hymenoptera: Eupelmidae). The former species is the most common parasitoid encountered in native *Agrilus* populations. The second species, itself an alien, probably arrived in North America from Asia on some host other than EAB because it was first encountered in 1994 in Virginia. Both species were reported during the Michigan study. Subsequent trapping at this Ontario location using sticky bands suggested a parasitism rate of ~40% by *P. sulcata*. Preliminary information on the distribution, ecology and phenology of these parasitoids are presented. These parasitoids are being evaluated for their potential for augmentative and/or inundative control of the borer.

PRELETHAL EFFECT OF ENTOMOPATHOGENOUS FUNGUS *BEAUVERIA BASSIANA* (BALS.) VUILL. OVER EUROPEAN PAPER WASP *POLISTES DOMINULUS* (HYMENOPTERA: VESPIDAE)

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The prelethal effect of an infection is an aspect of entomopathogenic fungi efficacy that does not include insect mortality but affects or decreases various aspects of a host's biology. The objective of this research was to evaluate prelethal effect of Qu-B933 isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes) over *Polistes dominula* (Christ) (Hymenoptera: Vespidae) wasps. A matrix was build to establish activities and distribution of time on healthy wasp colonies, comparing with wasps belonging to colonies receiving a liquid bait with a dose of 10^8 espores mL⁻¹ using The Observer™ software and grouping activities in active and inactive times. The test results showed significant decreases ($P=0.05$) in

total active time of inoculated wasps, reaching a 79.89% decrease at day 11 of test. The isolate application caused: a progressive reduction in activities such as drinking, washing, nest expansion, larva feeding, flying, walking, and interaction with other individuals; increased inactive time in inoculated wasps, finally causing insect death from the 10th day; and collapse of inoculated colonies due to a lack of defense work, cleaning and nest maintenance.

CONSERVING AND USING ENTOMOPATHOGENIC FUNGI AND NEMATODES WITHIN CHILE

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The Insect Pathology Program at INIA Quilamapu (Chile) is working in collaboration with CABI (UK) on a Darwin Initiative (DEFRA-UK) to conserve and use entomopathogenic microorganisms in Chile. The aim is to collect entomopathogenic fungi and nematodes from some of the major ecological habitats in Chile. Seven survey transects have been chosen: 1. Latitude 20°, with sections of Altiplano and on the periphery of the Atacama desert; 2. Latitude 30°, desert with remnants of ancient tropical forests; 3. Latitude 33°, an area of Mediterranean vegetation; 4. Latitude 37°, a transitional zone from dryland into wetland; 5. Latitude 46°, heavy rainfall, relatively cold, with humid forests and areas of pampas, 6. Latitude 52°, Tierra del fuego, with near Antarctic conditions and flora and fauna adapted to low temperatures. The transects have been surveyed, revealing 457 isolates of entomopathogenic fungi, predominately *Metarhizium* and *Beauveria* spp. (Hyphomycetes) and 99 isolates of nematode, *Heterorhabditis* (Rhabditida: Heterorhabditidae) and *Steinernema* spp. (Rhabditida: Steinernematidae). The isolates will be placed into the Genetic Resource Collection at INIA, significantly enhancing the bank of indigenous germplasm already present. It is likely that indigenous isolates will show stronger adaptations to conditions in Chile compared to exotic isolates and could be important pest control options.

EFFECTIVENESS OF *ASECODES HISPINARUM* AS AN INTRODUCED CONTROL AGENT OF THE INVASIVE PEST, THE COCONUT HISPINE BEETLE IN VIETNAM

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The coconut hispine beetle, *Brontispa longissima* Gestro (Coleoptera: Chrysomelidae) is one of the most serious pests of coconut palms in Southeast Asia. The beetle is believed to be native to Indonesia and Papua New Guinea, and recently it was accidentally introduced into continental Southeast Asian countries. In Vietnam, *B. longissima* was found in the Mekong Delta region (Southern Vietnam) in 1999 and then spread to the central region. Almost all the coconut trees in the southern and central parts of Vietnam were heavily infested with this beetle by 2003. The larval parasitoid *Asecodes hispinarum* Boucek (Hymenoptera: Eulophidae) was introduced from Western Samoa in 2003 with the support of FAO, and was released to those areas of Vietnam. The beetle was no longer abundant in the southern part of the country in 2007; 10-40% of trees were still infested with the beetle, but only less than 20% of leaves in the infested trees were damaged. This result suggests that the introduced larval parasitoid succeeded in controlling the beetle. On the contrary, in the central part of Vietnam such as Phu Yen and Khanh Hoa provinces, there were still many trees with heavy infestations found, although the parasitoid had been released. We discuss factors responsible for the difference in effectiveness of biological control between the two regions in Vietnam.

ARTIFICIAL DIET OF THE COCONUT HISPINE BEETLE *BRONTISPA LONGISSIMA* FOR REARING ITS PARASITOID *ASECODES HISPINARUM*

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The coconut hispine beetle, *Brontispa longissima* Gestro (Coleoptera: Chrysomelidae) is one of the most serious pests of coconut, *Cocos nucifera* L. (Arecaceae) and several ornamental palms in Southeast Asia. The pest is believed to be native to Indonesia and Papua New Guinea, and recently it was accidentally introduced into continental Southeast Asian countries. The endoparasitoid *Asecodes hispinarum* Boucek (Hymenoptera: Eulophidae) specifically attacks larvae of *B. longissima* and could potentially be used as a biological control agent. To keep colonies of the wasp and the beetle in the laboratory, it is necessary to provide coconut fronds for the beetle, which are important parts for healthy growth of the trees and are relatively expensive even in Southeast Asia as well. We developed an artificial diet for *B. longissima* for a convenient rearing technique, which can maintain this beetle during the developmental stage from hatching to adult emergence with 41% survival. Females of *A. hispinarum* actively oviposited in 4th instar larvae of beetles reared on the diet. Of the oviposited hosts, 75% were mummified and 42% produced adult wasps.

POPULATION DYNAMICS AND ON-FARM FRUIT FLY INTEGRATED PEST MANAGEMENT IN MANGO ORCHARDS IN THE NATURAL AREA OF NIAYES IN SENEGAL

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The trend of the population of fruit flies follows the dynamic of the rains. This tendency is more perceptible in *Bactrocera invadens* Drew, Tsuruta & White than in *Ceratitis cosyra* (Walker) (Diptera: Tephritidae). From 350 individuals captured per trap, *B. invadens* seemed to disrupt the presence of *C. cosyra* and the other related fruit fly species. Such behavior is probably due to an interspecific competition and could be the fact that *C. cosyra* dominated emergences from the incubated fruits of alternate host plants up to 87% even though *B. invadens* was observed. Integrated pest management (IPM) package was tested which included: (1) male annihilation using wood blocks soaked in insecticide (malathion 500 EC) and lure (methyl eugenol and terpinyl acetate); (2) two protein hydrolysate bait applications (Success Appat at 1 L/ha); and (3) sanitation (weeding and destroying of the collected fallen fruits by the following practices: using black plastic bags, burying in holes, burning on the ground surface and incinerating with a barrel transformed into incinerator). The aim was to control fruit flies in mango orchards. Results showed a control as an inferred improvement in fruit fly infestations in the treated plot up to 83% compared to the untreated. From the above the method to destroy collected fruits using a reinforced black plastic bag would be recommended for popular use. When we compare methyl eugenol to the home-made baits of grinded nutmeg and NET, a beauty cream, we found that methyl eugenol attracted *B. invadens* significantly. Methyl eugenol's half life is also significantly longer (5 weeks) than the ground nutmeg (less than 1 week) ($P=0.0109$; $t=9.4935$; $df=2$). No capture was recorded in the NET based trap. In case of lack of methyl eugenol, the ground nutmeg might be recommended as an alternative product to renew every week.

NATIVE TRICHOGRAMMA PARASITISM OF THE NEWLY INTRODUCED LIGHT BROWN APPLE MOTH IN CALIFORNIA, USA

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Native to Australia, the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) is presently established in several coastal areas of California, USA. The present study investigates the impact of native *Trichogramma* species (Hymenoptera: Trichogrammatidae) upon LBAM in California. Objectives of the ongoing project include the identification of *Trichogramma* species responsible

for parasitism, seasonality of parasitism, and the variability of parasitism in the field across a range of LBAM host plants.

POTENTIAL USE OF NATIVE ISOLATES OF ENTOMOPATHOGENIC FUNGUS FOR OBSCURE MEALY BUG (*PSEUDOCOCCUS VIBURNI* (MASKELL)) CONTROL

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The obscure mealy bug is an important plant pest for Chilean fruit production due to cosmetic fruit damage and its quarantine importance for export markets. At laboratory were tested 100 isolates of native entomopathogenic fungi, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes), on third instar mealybugs. Every two days during 31 days, the mortality and sporulation were evaluated. The selected isolate was sprayed on all developmental stages of the mealybug, to obtain the instars susceptibility and LC₅₀. This isolate was also mass cultured and sprayed in preliminary field trials. Significant differences were detected among the 100 isolates ($P < 0.05$), the Qu M984 isolate showed the best potential control of mealybugs (85% mortality and 80% sporulation). The second instars showed the greatest susceptibility to the fungi with LC₅₀ 1×10^4 /ml. The field trials (dose of 1×10^{12} /hectare) showed a mealybug population decrease of 70% as compared with the control. Therefore, the future emphasis will be to increase dose concentrations. Entomopathogenic fungi have an important potential for control of obscure mealybugs in commercial fruit production.

CHARACTERIZATION AND EVALUATION OF AN INDIGENOUS GRANULOVIRUS OF *PIERIS BRASSICAE* L. IN THE NORTH-WESTERN HIMALAYAS

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Cole crops are important cash crops in Himachal Pradesh situated in north-western Himalaya between 30°22' to 33°12' N latitude and 75°45' to 79°04' E longitude. Amongst a number of insect pests, cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) is the major constraint in their successful cultivation, making insecticidal application mandatory. The increased consumer awareness has made it obligatory to reduce the pesticide usage in vegetables, which however requires the alternative pest management strategies. A granulovirus of *P. brassicae* isolated from the high temperate region (Sangla: altitude 2580 mt.) was characterized and evaluated for its effective field usage against the pest. Partial nucleotide sequencing using a granulin specific marker revealed 365 bp sequence of PbGV and was analyzed using the clustalW programme. Local isolate had maximum homology

(76%) with *Phthorimaea operculella* granulovirus followed by *Choristoneura occidentalis* granulovirus (75%), whereas, minimum homology (39%) was observed with *P. rapae* granulin gene. Laboratory evaluation of PbGV alone and in combination with 5% solvent extracts of botanicals (*Artemisia brevifolia*, neem seed kernel and *Eupatorium adenophorum*) revealed the synergistic effect of botanicals. The lowest LC₅₀ values were recorded for PbGV+neem seed kernel extracts i.e. 2.16×10^7 , 7.12×10^6 and 1.15×10^6 OBs/ ml, respectively for aqueous, methanolic and petroleum ether extracts against IV instar larvae. Field studies revealed that PbGV @ 500 LE/ha was effective in managing *P. brassicae*, however, when combined with Bt and NSKE, effective dosage was 250LE/ha. UV protectants and phagostimulants were also evaluated to improve PbGV persistence and pathogenicity.

NATIVE PARASITIC WASPS (HYMENOPTERA: BRACONIDAE): A NEW TOOL FOR FRUIT FLY (DIPTERA: TEPHRITIDAE) MANAGEMENT IN AUSTRALIA.

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Inundative releases of Australian parasitoid species have resulted in improved management of fruit flies (Tephritidae) in several regions of the world. Despite this, and the importance of fruit flies as pests, inundative releases of parasitoids are not yet used in Australia. This paper will present preliminary results on the development of such an approach for use in Australia. Initial work comprises field surveys of areas in inland New South Wales to determine the identity of fruit fly parasitoid species present and levels of parasitism. In Australia, a number of hymenopteran parasitoids are known to target Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) including *Diachasmimorpha tryoni* (Cameron) and *D. kraussii* (Fullaway) and the introduced *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae). The extent of *B. tryoni* sharing parasitoid species with other native fruit fly species including island fruit fly, *Dirioxia pornia* (Walker) and wild tobacco fruit fly, *B. cacuminata* (Hering) (Diptera: Tephritidae) will be reported. Small-scale, laboratory assessment of the ease of culturing each parasitoid species, along with overseas reports, will allow selection of the superior species for more detailed studies. Results will be discussed in relation to optimal rearing techniques and strategies for release frequency, rate, location, timing and pre-release feeding of parasitoids and potential incorporation into pest fruit fly management programs.

COMPOSITION OF PREDATORY FAUNA DURING THE PEAK PESTICIDAL APPLICATION SEASON IN COTTON CROP

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A detailed study on the composition of the predator fauna from potential growing areas of Punjab was carried out in 2004-2005. Five productive sites of cotton growing belt were examined from June to August with outbreaks of sucking and chewing insects of all stages, high temperature and repeated application of insecticides. The cotton varieties grown in the five selected localities generally included the high yielding resistant varieties with overall average yield of 35-40 monds / acre. The predator composition recorded after the start of pesticide spraying revealed that in all the selected sites *Orius* sp. (Hemiptera: Anthocoridae) is the dominant biocontrol agent with total catches of 216 individuals followed by *Geocoris* sp. (Hemiptera: Geocoridae) (209) and *Chrysoperla* sp. (Neuroptera: Chrysopidae) (157). The predatory potential of *Chrysoperla* sp. is quite conspicuous as most of the trapped individuals were seen on places heavily infested with soft bodied stages of both chewing and sucking insects: whiteflies, jassids, thrips and bollworms. Similar population trends were recorded at 15 day intervals during the entire collection season. The average temperature noted during the collection period ranged from 38.36 to 43.16°C.

UTILISING NATURAL ENEMIES ALREADY PRESENT IN NEW ZEALAND TO ENHANCE BIOLOGICAL CONTROL FOR GREENHOUSE CROPS

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Currently only a small number of biological control agents are available for use in New Zealand for greenhouse crops. Growers identified this as a major impediment to the uptake of biological control programmes. Additional biological control agents are required for new invasive species such as the tomato/potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Psyllidae), western flower thrips, *Franklinella occidentalis* (Pergande), and intonsa flower thrips, *Frankliniella intosa* (Trybom) (Thysanoptera: Thripidae). More effective biological control agents are also required for the established greenhouse pest such as greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), and onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). Compliance with the regulations for importation of biological control agent from countries outside New Zealand is costly and takes a considerable amount of time. Many of the biological control agents used in other countries are generalist predators and it is unlikely that these could be imported into

New Zealand because of the threat they pose to the indigenous fauna. The difficulties of importing new organisms has led to a search in New Zealand for biological control agents for greenhouse crops. The southern ladybird, *Cleobora mellyi* Mulsant (Coleoptera: Coccinellidae), and a psyllid parasitoid, *Tamarixia* sp. (Hymenoptera: Eulophidae), have potential as psyllid biological control agents. Two new whitefly natural enemies have also been found, the parasitoid *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) and the predatory mirid *Macrolophus pygmaeus* Rmbur (Hemiptera: Miridae). Laboratory colonies of the predatory mite *Amblydromalus limonicus* (Garman & McGregor) (Acari: Phytoseiidae) have also been established. This mite has the potential to improve thrips control and give additional control of whitefly and psyllids.

BIOLOGICAL CONTROL OF THE INVASIVE LETTUCE APHID *NASONOVIA RIBISNIGRI* IN OUTDOOR LETTUCE BY INDIGENOUS PREDATORS IN NEW ZEALAND

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Lettuce aphid, *Nasonovia ribisnigri* Mosely (Hemiptera: Aphidae), was first detected in New Zealand on lettuce, *Lactuca sativa* L. (Asteraceae) in Christchurch in March 2002. Lettuce growers immediately found the new pest difficult to control using foliar insecticide applications because lettuce aphid prefers to feed and reproduce in the interior of the lettuce. Control options for the lettuce aphid, including foliar-applied insecticides, insecticide drenches, lettuce aphid-resistant cultivars and biological control, were investigated in 10 lettuce field trials in Pukekohe (South Auckland) quarterly from September 2002 to April 2005. Biological control agents, including the Australasian Tasmanian lacewing, *Micromus tasmaniae* Walker (Neuroptera: Hemerobiidae), and the native indigenous hoverfly, *Melanostoma fasciatum* Macquart (Diptera: Syrphidae), consistently gave effective control of lettuce aphid and other aphid species in the 3 spring trials (October–December). Although these predators failed to reduce the high lettuce aphid populations to acceptable levels in the summer trials (January–March), aphid numbers in the untreated controls were lower than in foliar-applied insecticide treatments at harvest. Only low numbers of lacewings and hoverfly larvae were found in autumn trials (April–June) and they were rarely found in the winter trials (July–September). These trials demonstrate the potential of indigenous biological control agents to contribute to the management of a new exotic pest.

RESEARCH ADVANCE OF *BACILLUS THURINGIENSIS* AGAINST PESTS AND PLANT DISEASES

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Bacillus thuringiensis Berliner (Bacillaceae) is well known as a high-effective bio-insecticidal bacterium. Few people realized about the character of *B. thuringiensis* for plant disease-control. During the last 20 years of development of *B. thuringiensis*, many new functions of were reported. In this paper, novel functions of *B. thuringiensis* to control phytopathogenic microorganisms are summarized. The first function is AHL lactonase, which can quench the quorum sensing signal molecular N-acyl homoserine lactones and in turn significantly silence *Pectobacterium carotovorum* (Jones) Waldee, emend. Gardan *et al.* (= *Erwinia carotovora* (Jones) Lehmann & Neumann) (Enterobacteriaceae) virulence and decrease the incidence of *P. carotovorum* infection. The second function is zwittermicin A, a linear aminopolyol antibiotic with high activity against the *Oomycetes* and their relatives, as well as the algal protists and certain Gram-negative bacteria. At the same time, zwittermicin A can greatly enhance the efficacy of *B. thuringiensis* insecticides. Finally, the potential of *B. thuringiensis* to control plant parasitic nematodes and some new progresses were introduced. Each function had some striking results and showed good promise for application. On the other hand, some results suggested that the control spectrum of *B. thuringiensis* can be widened and help to better understand the role of *B. thuringiensis* in the oil ecosystem.

EVALUATION OF ENTOMOPATHOGENIC FUNGI AS A BIOLOGICAL CONTROL AGENT OF *CAMERARIA OHRIDELLA*, AN INVASIVE PEST OF *AESCULUS HIPPOCASTANUM* IN EUROPE

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We evaluated the potential for using entomopathogenic fungi to control the horse chestnut leaf-miner, *Cameraria ohridella* Deschka et Dimic (Lepidoptera, Gracillariidae). In the first step, the occurrence of entomopathogenic fungi in soil samples collected in the vicinity of horse chestnut trees heavily infested by *C. ohridella* was surveyed using the adapted *Galleria*-bait method. Totally, 45.3% of *Galleria* larvae (n=3840) were found infected with entomopathogenic fungi (Deuteromycetes). Dominant species found were *Isaria fumosorosea* syn. *Paecilomyces fumosoroseus* (Wize) Brown et Smith (77.6%) and *Beauveria bassiana* (Balsamo) Vuillemin (20.6%). *Isaria farinosa* (Holm ex S.F. Gray) Brown et Smith and *Metarhizium anisopliae* (Metschnikoff) Sorokin occurred rarely (1.7% and 0.1%, respectively). In addition, we discovered spontaneous infection of *C. ohridella* hibernating pupae by *I. fumosorosea* and *B. bassiana*. Both species were

successfully isolated from the host and cultivated on artificial medium. All isolated strains are deposited in the CCEFO (Culture Collection of Entomopathogenic Fungi Olesna) in the Czech Republic. The laboratory bioassays revealed a high insecticidal activity of *I. fumosorosea* blastospores and conidia on both *C. ohridella* pupae and eggs. Further research is currently on-going where we evaluate selected strains of *I. fumosorosea* in field trials. Ways of implementation of entomopathogenic fungi in the IPM of *C. ohridella* are discussed.

SESSION 4

MOLECULAR TOOLS IN BIOLOGICAL CONTROL

DIVERSITY OF PROKARYOTIC ENDOSYMBIONTS ASSOCIATED WITH THREE BIOTYPES OF *BEMISIA TABACI* AND THEIR PARASITOIDS IN CHINA

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Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is composed of numerous biotypes, which may differ from each other both genetically and biologically. In China, biotypes B, Q and some indigenous biotypes like CV have been reported. *Bemisia tabaci* harbors primary endosymbionts, *Portiera aleyrodidarum* Thao & Baumann (Halmonadaceae) as well as several secondary endosymbionts including *Wolbachia* (Anaplasmataceae) and *Arsenophonus* (Enterobacteriaceae). Endosymbiont infection frequencies in laboratory populations of three *B. tabaci* biotypes; B, Q and CV were determined by PCR in order to test for correlation between bacterial composition to biotypes. *P. aleyrodidarum* and *Wolbachia* were detected in populations of the all three biotypes, while *Arsenophonus* was found in the Q and B biotypes. Overall infection by *P. aleyrodidarum* was 52%. While in secondary endosymbionts, *Wolbachia* was found to be relatively more abundant with an overall whitefly infection of 58% and *Arsenophonus* infection was 27%. Moreover, we revealed that various parasitoid species of *Eretmocerus* and *Encarsia* (Hymenoptera: Aphelinidae), used as biological agents of *B. tabaci*, were also infected with *Wolbachia*, with an overall infection level of 49.1%. A phylogenetic tree using the *wsp* gene of *Wolbachia* clearly indicated that all the *Wolbachia* sequences from *B. tabaci* populations shared close relationships with those from parasitoids, which may reflect the co evolution of the *Wolbachia* populations in *B. tabaci* and parasitoids. No differences in the primary endosymbionts were found among these three biotypes but in the secondary endosymbionts, differences in infection frequencies and the absence of *Arsenophonus* in the CV biotype suggest a possible contribution of these bacteria to host characteristics, which could be utilized for designing the suitable biological control of *B. tabaci*.

GENETICS OF BIOLOGICAL CONTROL: THE DIVERSITY OF *TERETRIUS NIGRESCENS*, PREDATOR OF THE LARGER GRAIN BORER, *PROSTEPHANUS TRUNCATUS*

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The larger grain borer, an alien invasive postharvest pest, has spread to all regions of Africa since its introduction two decades ago, becoming the single worst insect pest in all regions. Biological control was considered a key tool in the integrated management of the pest and two populations of *Teretrius nigrescens* Lewis (Coleoptera: Histeridae), recovered from Costa Rica and Mexico, were released in several areas in Africa between 1991 and 1996. However, the effectiveness of the predator is inconsistent in various areas, with greater successes in hot-humid areas. To establish the possible causes of this variability, we investigated the genetic diversity among populations of the predator recovered from various agroecological zones in Mexico and Costa Rica. Molecular analysis based on cytochrome oxidase 1 (COI) and Internal Transcribed Spacer (ITS) sequences has shown that the two populations released in Africa are distinct and can be separated by PCR-RFLP analysis. Variation in CO2 involved nucleotide substitutions but was not informative. Environmental conditions, especially temperature, altitude and climate seem discriminant in determining strain distribution. For instance, lowland populations clustered together despite geographical distance between them. It is therefore possible that there exist ecological or geographical types of this predator, specialised to different environmental conditions. A simple molecular identification procedure has been proposed for possible strain-specific monitoring of the predator populations.

CAN A *BEAUVERIA BASSIANA* F418 TRANSFORMANT EXPRESSING GREEN FLUORESCENCE BE USED TO MONITOR THE FATE OF THIS ENTOMOPATHOGENIC FUNGUS IN SOIL?

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The clover root weevil (CRW), *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae), is a major pest of white clover, *Trifolium repens* L. (Fabaceae) in New Zealand pastures. The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes) F418 strain showed good efficacy against CRW in the laboratory but control and establishment was variable in field trials. To devise better use practices for this fungal biopesticide, a better understanding of the ecology and population dynamics of the pathogen in soil is needed. A *B. bassiana* F418 transformant (*gfp* F418 tr1) containing green fluorescent and hygromycin resistance genes was created for this purpose. Prior to testing in soil, however, the characteristics of the transformant need to be assessed and compared to the wild type to ensure that key characteristics have been maintained following insertion of the genes into the fungal genome. Clonal populations of *B. bassiana* F418 and *gfp*

F418 tr1 were used in this study. *In vitro* tests included a comparison of germination rate, colony growth, conidial yield and virulence towards *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). No differences between the two strains were detected. Persistence of the two strains was evaluated in sterile soil under different abiotic factors. Again, no significant differences between the two strains were detected. *In vitro* and *in vivo* experiments confirmed the similarity of the transformant strain *gfp* F418 tr1 with the wild type. As a result, the transformant *gfp* F418 tr1 can be used to study the fate of *B. bassiana* in soil.

CHARACTERIZATION OF A NOVEL ENDONUCLEASE OF *DROSOPHILA* RETROTRANSPOSON AND ITS APPLICATION TO MOSQUITO CONTROL

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An endonuclease domain encoding 323 amino acids in the ORF2 of TART retrotransposable element of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) was cloned and over-expressed in *E. coli*. The mutant counterparts including one containing H230A point mutation and two truncated forms were also constructed and over-expressed for purification. All the recombinant proteins were purified to homogeneity. The wild-type form was shown to exhibit both AP-endonuclease and endonuclease activities using supercoiled plasmid DNA as the substrate. In contrast, the point-mutation and truncated forms are inactive, even in a more than 10-fold larger amount of protein than the wild-type. The activity can be stabilized in 30% glycerol at -20 °C for at least 2 months. The preferred hot-spot DNA cutting sites are also characterized. Since TART is responsible to transpose to broken chromosome ends, the endonuclease activity of ORF2 may well play an essential role of telomere maintenance. Furthermore, from BLAST search, two species of mosquitoes, *Culex pipiens* L. and *Anopheles gambiae* Giles (Diptera: Culicidae) are found to also contain the homologous endonuclease domain in their genome. Thus transgenic strategies can be designed based on the endonuclease domain to either enhance the endonuclease activity in order to cleave the telomeric DNA, or to knock down the nuclease activity by RNAi in order to spoil the telomere maintenance function in *A. gambiae*, *C. pipiens*, as well as *Drosophila*. Our results will be able to provide a novel way in controlling these Diptera species.

DIFFERENTIAL GENE EXPRESSION OF THE FAT BODY TISSUE OF *DIATRAEA SACCHARALIS* PARASITIZED BY *COTESIA FLAVIPES*

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Fat bodies are the most important tissue for protein synthesis and storage in insects, besides its role on many other metabolic processes. Because of its role in insect metabolism, this tissue is one of the main targets for regulation by koinobiont parasitoids. Parasitoids regulate their host by inducing changes in many host physiological processes, such as protein synthesis, hormone synthesis and degradation, as a way to control host growth and development to benefit the parasitoid immature development. *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) has been shown to induce changes in the hemolymph protein profile of the host, the sugarcane borer *Diatraea saccharalis* (Fab.) (Lepidoptera: Crambidae), indicating this parasitoid may be regulating the host protein synthesis at the molecular level. Therefore, we analyzed the gene expression profile of the host fat body by DD-PCR at different stages of parasitoid development (1, 3, 5, 7 and 9 d after parasitization). DD-PCR analysis revealed that several transcripts were regulated during parasitoid development, including a storage protein, a methionine-rich protein. In this presentation, we will provide information on the regulation of gene expression of the host fat body by *C. flavipes* and on the cDNA encoding the methionine-rich protein differentially expressed in parasitized hosts.

DEVELOPMENT OF GROUP-SPECIFIC PRIMERS FOR PREY DNA DETECTION IN THE PREDATOR GUT

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Molecular techniques to analyze the gut content of polyphagous predators allows understanding of how the diversity and the availability of alternative prey (non-pest) contribute to the maintenance of predator populations in the crop. The Order of springtails (Hexapoda: Collembola), covers a whole range of species present in the soil of agricultural ecosystems, and represents one of the most abundant alternative prey. Some arthropod species have been described as major predators of springtails in unfavorable periods (no pest species present). In these periods the springtails represent in many cases the numerically dominant alternative prey. Group-specific primers were designed for the first time in order to detect springtails within predator gut contents. Degenerate primers were developed based on the sequences of 17 species of springtails collected in agricultural ecosystems. 18S rDNA was the region used to be a multi-copy and semi-conserved genomic region. Primers designed amplified DNA fragments of 272 and 177 bp. Specificity analysis demonstrated that these markers were not detecting DNA of 41 other species of arthropods. The use of these group-specific primers is advantageous when studying a broad spectrum of species close phylogenetically and/or potentially ingested by a polyphagous predator. These group-specific markers can greatly facilitate the study of trophic relationships between springtails and generalists predators.

POST-RELEASE TRACING OF AUGMENTATIVELY RELEASED *ORIUS STRIGICOLLIS* (POPPIUS) (HETEROPTERA: ANTHOCORIDAE) BY DNA MARKERS

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Orius strigicollis (Poppius) (Hemiptera: Anthocoridae), a predator of thrips and other small insect pests of fruit vegetables, is now commercially produced and widely used in greenhouses in Japan. However, it is a native species and closely related species are also distributed in the field, and it is impossible to morphologically distinguish between field and commercial strains of *O. strigicollis*. As a consequence, it is difficult to evaluate the effectiveness of released *O. strigicollis* in biological control programs. In order to overcome this problem, we developed two DNA marker systems: multiplex PCR to distinguish *Orius* species, and microsatellite markers for *O. strigicollis*. Using the multiplex PCR, we can easily distinguish five native *Orius* species in Japan. Using the microsatellite DNA markers, we can distinguish between field and commercial strains of *O. strigicollis*, since the allele frequencies and genetic diversities of these strains are remarkably different. Using these two methods, we demonstrated that the *Orius* bugs that remained in greenhouses after harvest were descended from individuals of the released strain.

THE INSECT MIDGUT AS A TARGET FOR MICROBIAL TOXINS – THE CASE OF AMBER DISEASE IN THE NEW ZEALAND GRASS GRUB

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Amber disease of the endemic New Zealand grass grub, *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae), is caused by strains of the bacteria *Serratia entomophila* Grimont *et al.* and *S. proteamaculans* (Paine & Stansfield) Grimont *et al.* (Enterobacteriaceae) containing a specific plasmid (pADAP), which encodes antifeeding and gut clearance genes. Ingestion of pathogenic bacteria produces a cascade of disease effects centred around changes in the grass grub larval midgut. These include cessation of feeding, expulsion of frass, an amber colouration associated with rapid clearance of the midgut contents, a near elimination of activity of the major midgut digestive enzymes (serine proteases); and eventual death of larvae. In an effort to gain a more detailed understanding of the processes involved during disease induced changes to the midgut, we have employed a range of new methods from the genomic and proteomic fields. Results from quantitative PCR (qPCR) experiments on midgut epithelial cells have demonstrated that serine protease genes are not down regulated following infection, even though midgut proteolytic enzyme activity is essentially eliminated following infection. Proteomic analysis reveals an increase in protein accumulation in gut cells from amber diseased *C. zealandica*. These results suggest that disease effects serine protease formation and transport rather than transcription. The disease appears to target serine protease production as α -amylase continues to be synthesized in the midgut of diseased larvae. While histology shows no obvious gross cytotoxic effects on the midgut cells, other cellular phenotypes are currently under investigation. The results suggest that the insect midgut will provide novel targets for biological control and a model for the mechanism of amber disease mode of action in *C. zealandica* will be presented.

OPTIMIZATION OF MOLECULAR METHODS TO DETECT INGESTED PLANT DNA WITHIN INSECTS

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Molecular techniques are very useful methods to study trophic relationships between pests and their natural enemies. However, many natural enemies are also known to profit from plant-provided resources. Therefore, the identification of plant specific DNA in the gut of different insects would allow us to identify which plant species the insect has been feeding on and thus, be contributing to its conservation in the agricultural landscape. This study discusses the detection of plant DNA in the gut of several predators and pest species using different plants. For this purpose specific primers were designed from the ITS 1-2 (*Internal Transcribed Spacer 1-2*) region. ITS primers allowed the amplification of DNA plant fragments within the gut of the insect species tested but not in all cases. Results are discussed in relation to the ingested plant, the feeding physiology of the insect (sucking or chewing) and the sensitivity of the protocol used.

SPECIES-SPECIFICITY OF A PCR-BASED ASSAY FOR *CERATITIS CAPITATA* (WIEDEMANN) PARASITOIDS

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The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is a devastating fruit pest worldwide due to its global distribution, wide range of hosts, rapid dispersion and adaptation to low temperatures. The demand for insecticide-free fresh fruit is encouraging the use of environment-friendly control methods such as the sterile insect technique (SIT) and biological control programs with parasitoids. Accordingly, current area-wide medfly control program in Spain included both SIT and parasitoids releases. The imported braconid species *Diachasmimorpha tryoni* (Cameron), *D. longicaudata* (Ashmead), and *Fopius arisanus* Sonan (Hymenoptera: Braconidae) are reared at IVIA facilities and releases to control *C. capitata* are planned to start in autumn 2008. Accurate identification of natural enemies is the cornerstone of biological control. Currently the identification of parasitoids and estimation of parasitism rates rely on tedious and time-consuming dissection and rearing methods. The state-of-the art for estimating parasitism rates requires the use of molecular techniques. The present study describes species-specific PCR primers, based on the ribosomal internal transcribed spacer 1 region (ITS1) sequence, for the three species of medfly parasitoids reared at IVIA facilities, and their use in a multiplex PCR assay. Results indicate that the primer set amplify uniquely sized species-specific PCR products in a single reaction allowing determination of the parasitoid species and the parasitism rate for each medfly larvae or pupae tested.

USING MOLECULAR GUT CONTENT ANALYSIS TO TRACK DIETARY CHOICES IN SOIL INSECT PESTS

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Wireworms, the larvae of click-beetles (Coleoptera: Elateridae), are highly abundant herbivores in grassland and arable land and can cause considerable damage to crops. Besides crops, weeds are known to be regularly fed on by these soil insects and they might be used to reduce wireworm feeding pressure on crops. As wireworms consume their food in a liquid state, conventional approaches of prey detection do not allow studying their food choices. Moreover, plant roots are hard to identify morphologically on a species-specific basis, which further hampers assessment of below-ground herbivory. Here we present a PCR-based approach to identify plant material in the guts of click beetle larvae. *Agriotes obscurus* (L.)

(Coleoptera: Elateridae) arvae were fed with wheat, *Triticum aestivum* L. and maize, *Zea mays* L. (Poaceae) and batches of 10 individuals each frozen at different time points post feeding. Species-specific PCR primers were developed to amplify short DNA fragments of wheat and maize. The outcomes of this experiment will tell for how long post feeding plant DNA can be amplified from their wireworm consumers. Furthermore, first results on the application of this approach to the analysis of wireworm food choices in arable fields and grasslands will be presented.

TRFLP REVEALS THE DIET BREADTH OF ARTHROPOD PREDATORS

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It is well recognised that arthropod predators may have an important role in biological control. However, it has been difficult to assess the importance of arthropod predators in pest control, partly because they have a reputation as generalists, which may not specifically suppress pests, and partly because the true breadth of their diets is largely unknown. We have been developing a method to analyse terminal restriction fragment length polymorphisms (TRFLP) as a tool for elucidating the diets of predators. This method has the potential to reveal all the recent prey species in a predator's diet a single biochemical analysis. We demonstrate the use of TRFLP the diet of adult *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) feeding on prey in broccolini crops in South Australia.

SESSION 5

GMO's AND BIOLOGICAL CONTROL

DEVELOPMENT OF *BACTROCERA CUCURBITAE* (COQUILLET) FIRST INSTAR LARVAE IN RESPONSE TO APPLICATION OF TRYPSIN INHIBITOR FROM LIMA BEAN

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Despite the pivotal role played in increasing the food and fiber production to meet the needs of the expanding population, chemical pesticides have led to pollution of the environment, development of pesticide resistance, pest resurgence and destruction of natural biocontrol agents. Consequently, many ecofriendly approaches are being developed and assessed for the control of insect pests during the last two decades. Among these the biopesticides are supposed to cause least disruption to agroecosystem thus helping to maintain ecological balance while keeping the population of pests below economic threshold levels. Recently, the exploration of plant defensive proteins as pest control agents is gaining quite some attention. One such class of defensive proteins is protease inhibitors. Adverse effects of plant protease inhibitors on the physiology and development of some insect pests have been reported. The present investigation was undertaken to study the influence of Trypsin inhibitor from lima bean (LbTI) on the development of first instar larvae (48-56 hr) of melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). The melon fruit fly is an economically important pest of vegetables and fruits in the tropical countries. The first instar larvae were given ad libitum feeding on artificial diet containing LbTI of different concentrations (4, 16, 64 and 256 ppm) and a control diet. The larval period and total development period showed significant ($p < 0.01$) prolongation in the treated concentrations as compared to control. The larval period increased by approximately 21% in 256 ppm treatment and total development period increased by 21.5%. However, the inhibitor had no significant effect on the pupal period. The percentage pupation and adult emergence significantly ($p < 0.01$) declined to 58.335 and 39.28% at 256 ppm, respectively, as compared to their respective controls. On the basis of adult emergence a very low LC_{50} value of 153 ppm of LbTI was calculated with probit against *B. cucurbitae*.

BIOLOGICAL CONTROL OF *ZABRUS TENEBRIOIDES* BY SEED EXTRACT OF *MELIA AZADERACH*

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The insecticidal properties of a number of plants have been discovered long ago, and some of the plants can compete with synthetic means of control. *Melia azedarach* L. (Meliaceae) belongs to a group of plants for which insecticidal properties have been known for quite a long time. The purpose of the present study was to evaluate of insecticidal effect of seed extract of *M. azaderach* against *Zabrus tenebrioides* (Goeze) (Coleoptera: Carabidae), that is an important pest of Wheat. Extractions were applied (doses, 25% and 50%) on egg, larvae, pupa and adult insects. The present experiments showed a significant increase in mortality of egg, larvae, pupa and adult insects.

SESSION 6

IMPACT OF LANDSCAPE COMPOSITION AND STRUCTURE ON NATURAL ENEMIES

SPATIAL ASPECTS OF THE BIOLOGICAL CONTROL OF THRIPS IN VINEYARDS

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Thrips obscuratus (Crawford) (Thysanoptera: Thripidae), the endemic New Zealand flower thrips, is the most widespread of the New Zealand thrips. It has colonised many introduced European plants, including grapes, and numbers in vines can reach 2 million/hectare in spring. On grapes, thrips can cause injuries to the pedicel and scarring of the berry skin. As well as being unsightly, the initial punctures likely provide entry points for fungal diseases such as *Botrytis cinerea* (Persoon) Fries (Hyphomycetes: Sclerotiniaceae). This disease can cost New Zealand's wine industry US\$10million per year in lost revenues, as well as control costs. When grapes flower in the spring, thrips disperse into the vineyards from the flower sources around them and from distant over-wintering sites. Thrips can disperse on prevailing winds over large distances and high numbers can move into a vineyard over a short time. They feed and reproduce on the grape flowers and berries. Landscape composition and habitat management of agro-ecosystems are important factors that may contribute to sustainable pest management. The mechanisms of this are not well understood. Understanding how the landscape plays a role in determining thrips populations around Waipara Valley (Canterbury, New Zealand) vineyards is important for biological control and management of this pest in this area. Neighbouring agricultural areas and non-crop vegetation may play a role in thrips density and in consequent damage to nearby vineyards. The landscape may be providing alternative host plants that thrips overwinter on, facilitating their immigration into the vineyard in spring. Natural enemies of thrips may be encouraged into the area if desirable habitats are available. The aim of this project is to inform decision-making in vineyards so vine growers can successfully manage potential non-crop sources of this pest.

MIGRATION OF COCCINELLIDS TO ALFALFA FIELDS VARYING IN EDGE VEGETATION

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Field edges and adjacent vegetation can influence the migration of natural enemies to crops. More permanent and permeable edges, where natural enemies can overwinter or easily cross over, should enhance their early arrival. Edge orientation may also affect their arrival. In this study we evaluated the abundance and species richness of Coccinellidae (Coleoptera) associated with alfalfa, *Medicago sativa* L. (Fabaceae), crops with different edges: eucalyptus, mix of acacia, blackberries and poplar (F-B-P), vineyards, corn, and annual vegetables. Edges varied in composition, permeability, height and orientation. Three sticky traps (1.5 m²) placed 1 to 3 m above ground at borders, were sampled from September 2007 to May 2008. We collected 90,396 coccinellids from 13 species. Neither total abundance nor species richness were significantly affected by adjacent vegetation, but at species level *Psyllobora picta* Germar and *Cycloneda sanguinea* (L.) were more abundant in alfalfa adjacent to eucalyptus and F-B-P, *Olla v-nigrum* Casey, was more abundant in fields adjacent to vegetables and *Hyperaspis sphaeridioides* Mulsant in those next to vineyards. *Adalia deficiens* Mulsant preferred all, except fields next to vegetables. The temporal sequence of coccinellid species was similar in all alfalfa crops, independent of field edge. We used canonical correspondence analyses (CCA) to examine the relationship between coccinellid communities in alfalfa crops and environmental variables describing different edges types. Axis 1 (height) and 2 (orientation) combined explained 94% of the variation in species abundance and the environmental variables. In particular, *P. picta* was strongly influenced by border height, while *S. bicolor*, *A. deficiens* and *C. sanguinea* were moderately influenced. Conversely, *O. v-nigrum* was negatively affected by height. *H. sphaeridioides* was negatively affected by borders with a northern exposure. These results show that crop edges may affect the arrival of some coccinellid species, which should be considered in the design of agricultural landscapes to improve biological pest control.

THE EFFECT OF LOCAL AGROLANDSCAPE ON SPIDER ASSEMBLAGES IN AGROECOSYSTEMS

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The importance of spiders in controlling many dangerous pests is broadly recognized. Spider assemblages were studied in agroecosystems of two distant regions of the European part of Russia, which differ in agricultural landscapes. The fields of Moscow Area (M) are of medium size (10-15 ha) and surrounded by mixed forests. In Krasnodar Province (K) large fields (up to 100 ha) are separated by shelterbelts. Entomological sweeping and pitfall trapping were performed for six crop seasons. Plots were set in the cropland, viz. in field edges (FE) (10 m from the field border) and in the field centre (FC) (150 m in M, 200 and 400 m in K), as well as in grassy field margins (FM) and bordering strips of adjacent habitats. Taxonomic composition of dominant spider species in FC is not affected by the crop, surrounding landscape and field size and is similar to that in European agroecosystems. However, the abundance and spatial distribution of particular species, as well as their seasonal fluctuations, were largely determined by the crop characteristics and period of their

vegetation. Agrobiont species colonize the field regardless of its size. Taxonomic composition of dominant spider species in FM was affected both by adjacent natural habitats and by the flora of the margins. Some of the species penetrate into FE and become more abundant there than cropland-preferring species. However they do not contribute to the total spider population in the rest of the field, as they do not penetrate into the croplands beyond their edges.

LANDSCAPE MANAGEMENT FOR CONSERVATIVE PEST CONTROL: A CASE STUDY IN AVOCADO ORCHARDS

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Avocado, *Persea americana* Mill. (Lauraceae) is a fruit tree species that was introduced into Southern Spain from Central America during the XVIth century. Nowadays, it is the main subtropical crop with c.a. 40.000 ha. Its economic importance and acceptance in the European market relies on the fact that it is a chemical free product, because most local putative pests have been unable to affect the trees. However, in 2004 the perseia mite *Olygonychus perseae* (Tuttle, Baker & Abbatiello) (Acari: Tetranychidae), arrived, a species native to Central America that probably coevolved with avocados. In order to keep the ecological status of the Spanish avocados in the market, research is being carried out on pest biological control and conservative methods to enhance the colonization and persistence of natural enemies of the perseia mite in the avocado orchards. In the work presented here, we surveyed and identified to species level the natural enemies inhabiting both avocado trees and surrounding vegetation. Also, we explored the effect of laneways' vegetation cover on the abundance of natural enemies. Results suggested that cover vegetation may act as reservoir for natural enemies, and therefore may contribute to the biological control of the perseia mite on avocados.

SESSION 7

BIOLOGICAL CONTROL OF ARTHROPODS OF MEDICAL AND VETERINARY IMPORTANCE

USE OF *BACILLUS THUIRIGIENSIS* SOROVAR *ISRAELENIS* (BTI) IN LARGE SCALE CONTROL OF *AEDES AEGYPTI* BY THE BRAZILIAN NATIONAL DENGUE CONTROL PROGRAM

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Currently, dengue is one of the most important diseases affecting the Brazilian population and its control is mainly directed towards control of its vector, *Aedes aegypti* (L.) (Diptera: Culicidae). Social and environmental conditions, favorable to *Ae. aegypti* range expansion, led to the vector's dispersal since its reintroduction in 1976. This range expansion could not be controlled by the traditional methods employed for vector-borne diseases in Brazil and South America. Brazilian legislation regulates the selection, acquisition, and distribution of insecticides to be employed in dengue control. The National Dengue Control Program (PNCD) has used the organophosphate temephos for *Ae. aegypti* larval control since 1967. However, the continuous application of this product resulted in selection of resistant populations. In 1999, the Brazilian Health Ministry started the coordination of an integrated program (Rede Nacional de Monitoramento da Resistência de *Ae. aegypti* a Inseticidas, MoReNAa) designed to monitor *Ae. aegypti* insecticide resistance. Populations from all regions of Brazil were tested for temephos resistance. The results were important to define novel control strategies. At present, 285 municipalities employ Bti for larval control. Even though Bti efficacy has been confirmed by many studies, its application on a large scale in dengue control programs is pioneering in Brazil. This is an initiative of the Ministério da Saúde, through the National Dengue Control Program (PNCD), faced with the need for insecticide substitution due to the low effectiveness of temephos in some municipalities.

TARGETED DELIVERY OF MICROBIAL BIOLARVICIDES FOR CONTROL OF MOSQUITO LARVAE

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A novel microbial biopesticide delivery system was developed for targeting mosquito larvae that feed and/or orient in specific areas of a water column. A granular coating-based encapsulation and controlled delivery system that utilized Matricap[®] technology was developed to target mosquito (Diptera: Culicidae) larvae in surface (e.g., *Anopheles* spp.), subsurface, or both surface and subsurface areas (e.g., *Aedes*, *Culex*, *Ochlerotatus* spp.) of an aquatic habitat with single or joint-action formulations of biolarvicides (i.e., *Bacillus thuringiensis* Berliner var. *israelensis* and/or *Bacillus sphaericus* Meyer & Neide (Bacillaceae)).

Matricap[®] controlled-delivery technology was based on the use of one or more coatings composed of fatty acids, fatty alcohols, or esters of these products (e.g., plasticizers), with or without one or more polymer binders, agglomerating agents, or additional formulation aids to regulate the release rate and profile of biolarvicides from solid carrier matrices. Specific gravity, solubility, hydrolysis, melting point, biodegradation, and/or chemical reactivity interactions of the coating(s), carrier, and microbial agent(s) in an admixture were utilized to design specific compositions for pre-flood or aquatic mosquito-management applications. If the formulation components were properly matched, granules could release biolarvicides to target larval populations for prolonged periods. Controlled delivery of biolarvicides was shown to be functions of the type, concentration, and/or number of coatings incorporated into a solid composition. Slight variations in the ratio of two or three coatings in a complex were observed to significantly increase or decrease the duration of delivery from a granular matrix as well as alter the controlled-release profile and positional distribution of a microbial agent in an aquatic habitat.

SESSION 8

RECENT ADVANCES IN CONSERVATION BIOLOGICAL CONTROL

CONSERVATION BIOLOGICAL CONTROL OF LYGUS BUGS WITH NATIVE *PERISTENUS* SPECIES IN SOUTHERN ALBERTA, CANADA

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Lygus bugs are intermittent pests in field crops of the Canadian prairies and are managed with insecticides as no alternatives are available. The objectives of this study were to (1) determine the suite of *Peristenus* (Hymenoptera: Braconidae) species that attack lygus bugs in and around crops in southern Alberta, (2) obtain baseline data on the levels of parasitism, and (3) document the basic seasonal distribution of the parasitoid species. Three *Peristenus* species were reared from lygus nymphs: *P. carcamoi* Goulet, *P. broadbenti* Goulet and *P. braunae* Goulet. *Peristenus braunae* is rare in the first generation of lygus in southern Alberta although it is commonly caught in sticky cards in grassy field margins in early May and may be more common in *Adelphocoris* spp. (Hemiptera: Miridae) or other Miridae. By far the dominant species on the first lygus generation was *P. carcamoi* which was collected from early June into late July suggesting that occasionally it attacks the second generation at low levels. *Peristenus broadbenti* was collected from mid-July into early August from the second generation nymphs. Rates of parasitism from nymphal dissections ranged from zero up to around 60 % at some sites on some collection dates. Ongoing studies are focusing on quantifying the temporal dynamics of parasitism rates in alfalfa and canola grown adjacently to determine the potential for native *Peristenus* to reduce lygus bugs from reaching pest status in crops.

EMERGENCE, ACTIVITY, AND HABITAT PREFERENCES OF THE ROOT MAGGOT PREDATOR-PARASITOID, *ALEOCHARA BILINEATA*, IN CANOLA IN WESTERN CANADA

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Aleochara bilineata Gyllenhal (Coleoptera: Staphylinidae) is an important natural enemy of root maggots (*Delia* spp.) (Diptera: Anthomyiidae), which are

serious pests of brassicaceous crops in North America and Europe. Adults of *A. bilineata* feed on eggs and larvae of root maggots, and *A. bilineata* larvae parasitize *Delia* spp. pupae. Emergence and seasonal activity periods of *A. bilineata* in canola were well synchronized with the occurrence of pre-imaginal life stages of its principal hosts, *Delia radicum* (L.) and *Delia platura* Meigen, with beetle emergence beginning shortly after the onset of root maggot oviposition. Approximately 428, 493, and 455 degree-days (soil base 5.57°C) and 187, 189, and 180 Julian days were required for 50% emergence in 2003, 2004, and 2005, respectively. Activity density of *A. bilineata* increased as monocotyledonous and dicotyledonous weed biomass declined. Significant preferences for canola species were observed, with *A. bilineata* associated most frequently with *Brassica rapa* L. compared with *Brassica napus* L. (Brassicaceae). Our research suggests that improved management of root maggot infestations in canola through enhancement of populations of the *A. bilineata* predator-parasitoid could be accomplished by reducing weed infestations; however, such recommendations should consider other predators in the system and the role of weeds in reducing root maggot oviposition and damage.

GROUND BEETLES (COLEOPTERA: CARABIDAE) FAUNA AND DOMINANT SPECIES IN FRUIT ORCHARDS OF MAZANDARAN PROVINCE, NORTHERN IRAN

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Ground beetles (Coleoptera: Carabidae) are one of the largest and most successful families of beetles in the world that arose in the early Tertiary. They comprise more than 40,000 named species most of which are found in the tropics. More than 30% of species are arboreal, though, in general temperate species are terrestrial, most are also flightless and predatory. Carabids are powerful predators and have an important role in the biological control of pests. These insects are increasingly used as a taxonomic study group in biodiversity and as bio-indicators in monitoring or site assessment studies for nature conservation purposes. The fauna of these beneficial insects was studied in fruit orchards of Mazandaran province (Northern Iran) through 2004-2006. In total, 23 species were collected in different regions of Mazandaran province. Plastic pit fall traps, sweep net and light traps were the main methods for sampling. Additionally, faunistic survey, population dynamics and species diversity were studied too. Sampling indicated that the highest diversity was observed in Amol and Ghaemshahr regions, and the lowest diversity in Babolsar. Also the highest population density was observed in Ghaemshahr and Behshahr regions, and the lowest one in Babolsar and Noor. *Harpalus griseus* (Panzer) had the widest distribution and *Acinopus megacephalus* (Rossi), *Bembidion menetriesi menetriesi* Kolenati, *Carabus roseni* Reitter, *Chlaenius steveni* Quensel and *Merizomena grandella* Semenov had the smallest. Also, the highest population density was observed for *H. griseus* and *Harpalus fuscicornis* Menetries and the lowest density for *A. megacephalus*, *B. menetriesi*, *C. steveni*, *C. rhodoterena*, *M.*

grandella and *Z. spectabilis*, respectively. The results indicate that *H. griseus* and *H. fuscicornis* are the dominant species in the fruit orchards of Mazandaran province. Since there is a very diverse pest fauna in orchards of Northern Iran, the main agricultural site in Iran, the carabids have an efficient role in decreasing pest population density.

LADY BEETLES (COLEOPTERA: COCCINELLIDAE) OF IRANIAN COTTON FIELDS AND SURROUNDING GRASSLANDS

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Lady beetles (Coleoptera: Coccinellidae) are one of the powerful and dominant predators in cotton fields and also other agroecosystems. The predaceous coccinellids are linked to biological control more often than any other taxa of predatory organisms. The beneficial status of these organisms has a rich history that is recognized by the general public and biological control practitioners alike. The lady beetles are important natural enemies of pest species, especially whitefly, aphids, mealybug, scales and mites. The role of naturally occurring Coccinellidae in suppressing pest populations is significant but poorly documented in many pest management programs that purport to conserve natural enemies. The fauna of these beneficial insects was studied in cotton fields and surrounding grasslands of Iran through 2000-2006. In total, 42 species in 17 genera were collected from 20 different localities where included cotton fields. The list of collected species are below. *Adalia bipunctata* (L.), *Adalia decempunctata* (L.), *Anisostica bitriangularis* (Say), *Exochomus octostignatus* (Gebler) [= *Brumus octostignatus* (Gebler)], *Brumus undecempunctata* (L.), *Chilocorus bipustulatus* (L.), *Chilocorus stigma* (Say), *Clitostethus arcuatus* (Rossi), *Coccinella hieroglyphica* L., *Coccinella monticola* Mulsant, *Coccinella septempunctata* L., *Coccinella trifasciata subversa* LeConte, *Coccinella undecimpunctata* L., *Cryptolaemus montrouzieri* Mulsant, *Delphastus pusillus* LeConte, *Exochomus flavipes* (Thunberg), *Exochomus nigromaculatus* (Goeze), *Exochomus nigripennis* (Erichson), *Exochomus pubescens* Küster, *Exochomus quadripustulatus* (L.), *Harmonia conformis* (Boisduval), *Hippodamia convergens* Guérin-Méneville, *Hippodamia variegata* Goeze, *Nephaspis oculatus* (Blatchley), *Nephus biguttatus* (Mulsant), *Nephus bipunctatus* (Kugellan), *Oenopia conglobata* (L.), *Oenopia conglobata contaminata* (L.), *Propylea quatuordecimpunctata* (L.), *Rodolia fausti* (Weise), *Scymnus apetzi* Mulsant, *Scymnus arcticus* lablokoff-Khnzorian, *Scymnus flavicollis* Redtenbacher, *Scymnus frontalis* (Fabricius), *Scymnus levaillanti* (Mulsant), *Scymnus pallipes* Mulsant, *Scymnus subvillosus* (Goeze), *Scymnus syriacus* (Marseul), *Stethorus gilvifrons* (Mulsant), *Stethorus nigripens* Kapur and *Stethorus punctillum* (Weise). Of the collected species, 5 species including, *A. bipunctata*, *C. bipustulatus*, *C. septempunctata*, *E. flavipes*, and *E. pubescens* are cosmopolitan and are more abundant species in nearly all the Iranian cotton fields. Also, the three genera

Scymnus, *Exochomus*, and *Coccinella* with 8, 6, and 5 species, respectively are more diverse than others in Iranian cotton fields.

BIOLOGICAL CONTROL OF GRAM POD BORER, *HELICOVERPA ARMIGERA* (HUBNER) – A NOVEL APPROACH IN CHICKPEA ECOSYSTEM

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Biological control has special significance in chickpea ecosystem that leads to a harmonious and sustainable management of gram pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Field experiments were conducted for 5 years from 2002 to 2007 to make an assessment of the beneficial role played by natural enemies such as predatory birds, coccinellids, wasps, and tachinids in the chickpea ecosystem at ARS, Gulbarga, Karnataka, India. The study revealed that sunflower acted as the most suitable live bird perch in the chickpea ecosystem as it is a very fast growing plant and provided rigid support for alighting insectivorous birds right from the vegetative stage until maturity of the crop. The basic principle involved in the study is that most of the *H. armigera* larvae present on chickpea migrated to sunflower plants right (7.4 to 8.5 larvae/plant) from the vegetative stage until the pod filling stage of chickpea with the sunflower plant having one to two larvae on every leaf that are easily visible and accessible to insectivorous birds, predatory wasps, coccinellids and tachinid flies, an efficient parasitoid. The study concluded that sowing sunflower (100 g/ha) and sorghum (100 g/ha) along with chickpea seeds resulted in a higher grain yield for chickpea (10.21 q/ha), with additional yields for sunflower (1.16 q/ha) and sorghum (10-12 kg/ha). This resulted in the highest net returns (Rs. 24559/ha) and the B:C ratio (1:8.70) over profenphos 50EC @ 3.0 ml/l as the standard check (1:5.12) suggesting that this is a low cost, eco-friendly, easily acceptable and adoptable technology by farmers for managing the chickpea pod borer.

EFFECT OF HABITAT MANIPULATION ON THE BIOLOGICAL CONTROL OF AFRICAN RICE GALL MIDGE *ORSEOLIA ORYZIVORA* HARRIS AND GAGNÉ (DIPTERA: CECIDOMYIIDAE)

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Biological control of African rice gall midge, *Orseolia oryzivora* Harris (AfRGM) (Diptera: Cecidomyiidae) using *Platygaster diplosisae* Risbec (Hymenoptera: Platygasteridae), an indigenous natural enemy, has not given the desired results due

to late arrival of this parasitoid in the field. Hence, the current experiment was conducted to determine the influence of habitat manipulation in the conservation of *P. diplosisae* in alternative host, *Orseolia bonzii* Harris (Diptera: Cecidomyiidae) which infests *Paspalum scrobiculatum* L. (Poaceae) weeds, in the management of AfRGM.

The strategy adopted was to plant a 1 m border of *P. scrobiculatum* seedlings around the border of each plot early in the season, to be infested naturally by *O. bonzii* and parasitized by *P. diplosisae* before planting of rice inside each plot. The control plots were planted with only rice seedlings leaving the border without any weeds. A host transfer experiment was conducted in a screen house to ensure that *P. diplosisae* could transfer from the alternative host to the rice midge. The results indicated that rice fields with a *Paspalum* border had lower levels of AfRGM infestation due to earlier and higher parasitism and resulted in increased grain yield compared to the control plots. The results of the host transfer experiment indicated that *P. diplosisae* persisted in *O. bonzii* until *O. oryzivora* colonized the first rice crop and then transferred to the rice midge. These results were confirmed by the significant correlation recorded from the study, which justifies its economic usefulness. The results of the study therefore demonstrate the role of habitat manipulation in the management of AfRGM.

BENEFITS OF AUSTRALIAN NATIVE VEGETATION FOR CONSERVATION BIOLOGICAL CONTROL IN COTTON

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The negative effects of landscape simplification on biological control have been well documented. 'Unmanaged' and 'undisturbed' land uses are often identified as being positively correlated with in-crop density and activity of natural enemies. Patterns of disturbance such as harvest, fallow and pesticide application can reduce natural-enemy population build-up and persistence in agricultural landscapes. Thus, undisturbed areas, such as remnant vegetation and shelterbelts, may act as important 'habitat islands' in agricultural landscapes. We employed spatially specific landscape analysis with cost-distance tools and follow-up mark-recapture trials to quantify the benefits of Australian native vegetation for enhancing the density of several natural enemy taxa with cotton fields. Landscape analysis identified that the density of *Oxyopes* spp. (Araneae: Oxyopidae) within cotton crops was strongly correlated with greater areas of woody native vegetation within 120 m of cotton crops; and the density of *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae) within crops was strongly correlated with increased connectivity of woody native vegetation with 1500 – 3000 m of cotton crops. Mark-recapture work indicated *Oxyopes* spp. and *D. bellulus*, and the also the predator *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), move from native vegetation shelterbelts to cotton crops. Shelterbelts present great potential as a landscape-level habitat manipulation tool. As well as acting as a local resource for natural enemies and a source of migration into cotton crops, the long continuous linear nature of shelterbelts is ideally suited to enhancing habitat connectivity within agricultural landscapes.

A FLORAL-DERIVED COMPOUND ATTRACTIVE TO THE TEPHRITID FRUIT FLY PARASITOID *DIACHASMIMORPHA LONGICAUDATA* AND OTHER BRACONIDAE (HYMENOPTERA)

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Many adult hymenopteran parasitoids consume the nectar of flowering plants. Field studies identified plants attractive (*Lobularia maritima* (L.) Desv. (Brassicaceae)) and unattractive (*Spermacoce verticillata* L. (Rubiaceae)) to certain opiine braconids. Under laboratory conditions, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), a parasitoid of tephritid fruit fly larvae and representative opiine, responded in flight tunnels to *L. maritima* but not to *S. verticillata*. Volatile chemicals of the two flowers were collected and analyzed using capillary gas liquid chromatography and mass spectral analysis. Acetophenone was isolated from *L. maritima* but not from *S. verticillata*. In flight tunnels, *D. longicaudata* were exposed to 10 concentrations (doses) of acetophenone. Female parasitoids showed a significant attraction to several acetophenone doses, with concentrations of 25 and 50 ng the most attractive. No odor source, either floral or floral-derived, was attractive to male parasitoids. Reliable trapping systems for parasitoid species, particularly species such as *D. longicaudata* used for augmentative biological control, would be a valuable monitoring tool. The volatiles of some other flowers that attract Braconidae in the field have also been found to contain acetophenone. At the present time, there are few, if any, florally-derived synthetic lures for attracting hymenopteran parasitoids.

DO WILDFLOWER STRIPS ENHANCE PEST CONTROL IN ORGANIC CABBAGE?

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Within this project we assess whether wildflower strips and companion plants increase the control of cabbage pests *Plutella xylostella* L. (Lepidoptera: Plutellidae), *Mamestra brassicae* L. (Lepidoptera: Noctuidae) and *Pieris rapae* L. (Lepidoptera: Pieridae) by (1) naturally occurring parasitoids and predators and (2) mass-released

Trichogramma brassicae (Bezdenko) (Hymenoptera: Trichogrammatidae) parasitoids. Two organic cabbage fields were used for this study: adjacent to each field a wildflower strip was sown and companion plants (*Centaurea cyanus* L. (Asteraceae)) intermixed within the crop. Within each field ~15,000 *M. brassicae* eggs were placed out to determine the parasitism rates by mass-released *T. brassicae* and to assess the levels of egg predation. Over 1,000 lepidopteran larvae were collected and screened for hymenopteran and tachinid parasitoid DNA using a multiplex PCR assay. Invertebrate generalist predators (n=1,063) were collected for DNA-based gut content analysis. The wildflower strip had a significant positive effect on *M. brassicae* egg parasitism rates as rates increased 5-fold in the vicinity to the strip. Moreover, companion plants enhanced invertebrate predation on *M. brassicae* eggs. Both, the release of *T. brassicae* and the use of companion plants, however, did not significantly increase egg parasitism rates. The infestation of plants by caterpillars increased with distance to the wildflower strip and there was a trend of decreasing larval parasitism rates with distance to the strip. Currently the invertebrate predators are being molecularly analysed to assess predation on unparasitized and parasitized lepidopteran pests.

FLOWERS, SYRPHIDS AND CONSERVATION BIOLOGICAL CONTROL

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A Functional Agro Biodiversity (FAB) pilot on 400 ha of Dutch arable farms showed that during the last four years conservation biological control was sufficient to keep aphids in both potato and wheat fields well below threshold levels. In order to optimize flowering field margins for the conservation of natural enemies, the suitability of various flowers were studied as food source for the important group of aphidophagous hoverflies, more specifically *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae). Flower suitability was partly evaluated on the basis of flower preference in multiple choice tests, and adult longevity when flower species were provided separately. The two measures appear to be correlated. Large differences in flower suitability are observed even within plant families. Flower suitability is partly determined by the accessibility of the nectar by the short tongue of the syrphid, but pollen amount and quality plays a role as well. The relative importance of nectar and pollen for syrphids was determined experimentally. In the presence of aphids and honeydew (a stimulus for reproduction) pollen increases longevity for females (much less for males) both in the absence of and in the presence of sugars. Pollen is essential for female reproduction, but when pollen and nectar is provided during the 6-day pre-oviposition period only, females oviposit during the following 6 days or more, producing on average about 100 eggs, before reproduction declines. This research shows that for aphid biocontrol by syrphids, the right kinds of flowers have to be available, but that flowers need not to be visited on a daily basis.

AUGUMENTING INSECTIVOROUS BIRDS IN AGRO-ECOSYSTEMS AS A NATURAL BIOLOGICAL CONTROL APPROACH- THE INDIAN EXPERIENCE

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Birds and insects have the ability to fly. This predisposes insects to bird predation. In spite of birds playing an important role in limiting insects, birds have not been considered a biological control "input". India has a rich bird fauna and ample studies have been done to prove that insectivorous birds prey on important pests. The first notable study almost dates to a century ago when Mason & Leffroy (1912) published a memoir of Indian birds feeding on harmful insects, based on gut analyses. Subsequently, many studies have established the usefulness of insectivorous birds, and these are reviewed. Some of the important insectivorous birds are Bee-eaters, *Merops orientalis* Latham (Meropidae), the King Crow, *Dicrurus adsimilis* (Bechstein) (Dicruridae), [Aitkin 1904], the Goldenbacked Woodpecker, *Dinopium benghalense* (L.) (Picidae), the Maharatta Woodpecker (*Dendrocopos mahrattensis* (Latham) (Picidae) [Krishnaswamy & Chowhan 1956], the Adjutant Stork, *Leptoptilos dubius* (Gmelin) (Ciconiidae), the Bulbuls. (*Pycnonotus* spp. (Piconotidae), the Pitta, *Pitta brachyuran* (L.) (Pittidae), the Common Myna, *Acridotheres tristis* L. (Sturnidae) [Singh and Singh (1960)], the Painted Partridge, *Francolinus pictus* (Jardin & Selby) (Phasianidae), [Sharma (1964)], Wagtails, *Motacilla* spp. (Motacillidae), [(Toor and Ramzan, 1978), Chakravarthy and Lingappa (1978)]. Further, in a stomach analyses it was found that the Cattle Egret, *Bubulcus ibis* (L.) (Ardeidae), the Whitebreasted Kingfisher, *Halcyon smyrnensis* (L.) (Alcedinidae), and the Redwattled Lapwing, *Vanellus indicus* (Boddaert), (Charadriidae) to be very beneficial [David (1965), (Mukerjee 1971 – 1975)]. Earlier, Verghese and Sriharan (1993) reviewed the conservation campaigns and awareness initiatives which have led to the use of practices in Indian agriculture that help augment insectivorous birds in agro-ecosystems. The current status of how birds have been recognized as a useful component in agro-ecosystems is discussed. This Indian experience is useful as conservation biological control.

SESSION 9

OMNIVORY IN BIOLOGICAL CONTROL

OLIGIDIC DIET, NATURAL AND FACTITIOUS HOSTS ON THE FEEDING BEHAVIOR AND BIOLOGY OF *RHYNOCORIS* *MARGINATUS* (FAB.) (HEMIPTERA: REDUVIIDAE)

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Rhynocoris marginatus (Fab.) (Hemiptera: Reduviidae) is an important polyphagous, general predator in India. Feeding behaviour, nymphal developmental period, survival rate, sex ratio, and fecundity on a meat-based oligidic diet (OD), natural [*Spodopetera litura* (Fab.) (Lepidoptera: Noctuidae) - SL] and a factitious host (*Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) - CC) were tested under laboratory conditions. Results revealed that the feeding behavior was similar for OD and the prey species. In general, irrespective of the prey species and OD approach time gradually diminished, and both handling time and weight gain were gradually increased as the predator grew older. Minimum nymphal developmental period was observed in *S. litura* (41 – 43 days), followed by *C. cephalonica* (44 – 47 days) and OD (65 – 70 days). A similar trend was also recorded for weight gain. However, the nymphal survival rate was higher for *C. cephalonica* than *S. litura* and OD. The sex ratio was female-biased in all the three categories (0.62 – 0.79, 0.65 – 0.82 and 0.75 – 0.85 for OD, *C. cephalonica* and *S. litura*, respectively). Similarly, *C. cephalonica* enhanced the predator fecundity compared to *S. litura* and OD. We concluded that the meat-based OD can be utilized for rearing of this predatory bug and utilized in the pest management programme.

BENEFITS AND DAMAGE OF *NESIDIOCORIS TENUIS* IN TOMATO CROPS

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Nesidiocoris tenuis Reuter (Heteroptera: Miridae) is one of the most controversial omnivore dicyphines because of the unknown balance between the injuries it causes to tomato crops when feeding on plants and the benefits as a generalist predator. Field experiments showed that *N. tenuis* is an efficient whitefly predator whose population rapidly rose following the increase in whitefly density. On the other hand, it also produced necrotic rings on stems and leaves, and flower abortion. Necrotic rings and flower abortion rates were directly related to the abundance of *N. tenuis* and whitefly. However, the reduction on truss weight by fruit

abortion was compensated by increasing fruit size and weight. The expected yield loss and density thresholds were modelled in relation to *N. tenuis* and whitefly abundance.

PREDATION BY THE MIRIDS *NESIDIOCORIS TENUIS* AND *MACROLOPHUS PYGMAEUS* ON THE TOMATO BORER *TUTA ABSOLUTA*

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The tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), native to South America, is an important tomato pest which appeared in eastern Spain at the end of 2006. As a first step to discovering the extent to which two indigenous predators, *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) can adapt to this invasive pest, the prey suitability of eggs and larval instars of *T. absoluta* was evaluated under laboratory conditions. Both predators preyed actively on *T. absoluta* eggs and all larval stages, although they preferred first-instar larvae. Secondly, in a semi-trial both mirids were separately inoculated on tomato plants to evaluate their predation on *T. absoluta*. After its installation in the crop, *Nesidiocoris tenuis* was highly effective in controlling *T. absoluta* under these experimental conditions, with leaflet and fruit infestation reductions of up to 97% and 100%, respectively. *Macrolophus pygmaeus* was also effective on this new pest, although its efficacy was lower in comparison to *N. tenuis* (76% and 56% of leaflet and fruit infestation reductions). Our results demonstrate that both mirids can adapt to this invasive pest, contributing to their value as biological control agents in tomato crops. A challenge for future studies will be to investigate how both predators, especially *N. tenuis*, can be used in biological control programs targeting *T. absoluta*.

SESSION 10

THE ROLE OF THEORY IN GREENHOUSE BIOLOGICAL CONTROL

STORAGE OF ADULTS OF *ORIVS INSIDIOSUS* AT LOW TEMPERATURES

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Storage at low temperatures is an important step during the process of mass production and use of natural enemies, since it allows greater flexibility in the rearing, transport, and release of beneficial agents. However, under this storage process it is important that it not lead to a reduction in fitness and quality of the natural enemy. This study aimed to evaluate the influence of different storage periods at low temperatures on the reproductive capacity and longevity of *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). The effect of storage period on mated and virgin females were evaluated also. Storage periods were 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days at temperatures of 5, 8, 10, 12°C, under continuous scotophase. The daily fecundity of the predator was higher at 8°C (4.1 eggs) compared to the other temperatures evaluated. The highest longevity of *O. insidiosus* was also observed at 8°C (14 days). *Orius insidiosus* adults can be stored for up to 10 days at 8°C without compromising its fecundity and longevity. Stored mated females presented higher daily fecundity (4.8 eggs) compared to stored virgin females (2.8 eggs). The percentage of stored virgin females that didn't lay eggs was 26.8% and only 4.3% of the stored mated females didn't lay eggs. These results might helpful to plan the process by which the predator *O. insidiosus* is mass reared and transported to the sites where it should be released. The ability to store predators even for short periods is a key factor in developing augmentative biological control programs.

ANAGRYUS SP. NOV. NR. *SINOPE* AS A BIOLOGICAL CONTROL AGENT OF *PHENACOCCLUS MADEIRENSIS* IN GREENHOUSE ORNAMENTAL S PRODUCTION

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Anagrus sp. nov. nr. *sinope* Noyes & Menezes (Hymenoptera: Encyrtidae) was discovered and evaluated as a potential biological control agent of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), in Georgia, USA. A series of studies were conducted to determine the host specificity and host size preference of *Anagrus* sp. nov. nr. *sinope* and its interactions with the

hosts. When presented with six common pestiferous mealybug species of ornamental plants, *Anagyrus* sp. nov. nr. *sinope* attacked *P. madeirensis*, *Phenacoccus solani* Ferris, *Planococcus citri* (Rossi) and *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae). However, the parasitoid only oviposited and successfully completed larval development in *P. madeirensis*. The oviposition preference of *Anagyrus* sp. nov. nr. *sinope* for six developmental stadia was also investigated. The parasitoid successfully oviposited and developed in all nymphal and adult stadia (except the third- and fourth-instar males). The numbers of parasitoids that emerged from a single third-instar or adult female were significantly higher and the body size of these parasitoids were significantly larger. Ovipositor insertion by *Anagyrus* sp. nov. nr. *sinope* contributed to significant mortality in the first- and second-instar nymphs. Host feeding did not contribute to significant mortality in the hosts. Parasitism by *Anagyrus* sp. nov. nr. *sinope* inhibited reproduction of young and unmated female mealybugs but not the per capita fecundity of gravid or ovipositing adult female mealybugs. From the studies, *Anagyrus* sp. nov. nr. *sinope* has demonstrated its potential as a biological control agent of *P. madeirensis*. The next step is to conduct controlled greenhouse trial to investigate the parasitoid's efficiency.

EFFICACY OF *STEINERNEMA CARPOCAPSAE* AGAINST *BEMISIA TABACI* INSTARS

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Steinernerematid nematodes have been used successfully for the control of dipteran pests in mushrooms, glasshouses and nurseries. Foliar applications of *Steinernema feltiae* (Filipjev) (Steinernematidae) have shown good levels of control of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). However, *Steinernema carpocapsae* (Weiser) (Steinernematidae) is thought to be more effective than *S. feltiae* at infecting larval stages of pests, but this requires independent testing. This study aimed to determine the efficacy of *S. carpocapsae* against *B. tabaci* life stages. Verbena plants were infested with *B. tabaci* adults and incubated at $25 \pm 1^\circ\text{C}$, 65% RH, 16L:8D for 48 h. After this period the adults were removed. Plants were then divided into four groups (one for each host stage). Each group was incubated for the required period of time (1-3 weeks) to reach the appropriate instar. All four stadia were then subjected to a nematode suspension (10,000 *S. carpocapsae* IJ ml^{-1} with 0.02% of the non-ionic wetting agent Agral). Treatments were applied to run-off and resulted in approximately 160 *S. carpocapsae* IJ per cm^2 of leaf area. Following treatment, host plants were maintained at $20 \pm 1^\circ\text{C}$, 85% RH and a 12L:12D regime. After 72 h mortality of *B. tabaci* larvae was assessed. Significantly higher mortality of *B. tabaci* occurred on nematode treated plants than controls. Instars 2-4 were significantly more susceptible than the first. No significant difference between susceptibility of second and third instars occurred. Mortality of nematode treated first instars was no greater than that of the controls. Therefore, *Steinernema carpocapsae* can significantly reduce *B. tabaci* populations with second instars proving most susceptible.

ORIOUS TANTILLUS AGAINST SCIRTOTHRIPS DORSALIS ON CAPSICUM UNDER POLYHOUSE CONDITIONS IN INDIA

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The possibility to use the native predator *Orius tantillus* (Motschulsky) (Hemiptera: Anthocoridae) for control of natural infestation of *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) on capsicum, *Capsicum annuum* L. (Solanaceae) grown under polyhouse conditions was tested. Three trials were conducted during 2007 – 2008 on Capsicum F₁ hybrid Indira. Two different methods were studied: (1) release of the predator on a Unit area basis; and (2) release of a known number on a per plant basis, such as 2, 3, 5, and 10. Augmentative release of the predator commenced at weekly intervals from the first appearance of thrips. Thrips sampling was done both by counting and visual rating on 0 - 5 scale. A total of 10 plants were randomly selected per treatment each week for counting thrips and predators. In all the trials, thrips density and damage were significantly lower in the predator release treatments as compared to untreated control treatments. About a 50% immediate reduction in thrips density was observed in all the trials within a week after release of 1 predator per plant. Thrips damage was more severe in the second trial, which resulted in the complete death of plants in the untreated control. In the third trial, overall there was a 79.41%, 81.48%, and 90.90% reduction and 100% increase in thrips density observed in the 2, 3 and 5 predators per plant treatments and the untreated control, respectively in 8 weeks. The result indicates that thrips can be controlled effectively under polyhouse conditions on capsicum with the releases of the predator *O. tantillus* at the rate of two per plant.

EFFICACY OF GREENHOUSE BIOLOGICAL CONTROL BY GENERALIST PREDATORS *HIPPODAMIA VARIEGATA* (COLEOPTERA: COCCINELLIDAE) AND *MICROMUS TASMANIAE* (NEUROPTERA: HEMEROBIIDAE)

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Two studies in greenhouses were conducted to assess the effect of release rates on predation by generalist predators *Hippodamia variegata* L. (Coleoptera: Coccinellidae) and *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) against a common greenhouse pest. Separately, and for both predators, a 10x40 m greenhouse with 35 caged mature cucumber plants, *Cucumis sativus* L. (Cucurbitaceae), was established at Gosford Horticultural Institute (New South Wales, Australia). Fourteen day-old cucumber plants were exposed to 20 adult

Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae) for 14 days to allow establishment of a uniform pest population prior to predator release into the cages. The experiment used five randomised blocks of seven biocontrol agent release rates with a negative control of no biocontrol agents. Predators were released as egg clusters deposited on leaves of *Vicia faba* L. (Fabaceae) that were secured to the stem of the individual cucumber plants. Release rates were 5, 15, 20, 25, 30, and 40 eggs/plant, and one negative control. Cucumber plants were monitored twice weekly for 60 days and measurements taken for pest density, predator abundance and predator reproduction. The second study considered the efficacy of *M. tasmaniae* as a greenhouse biocontrol agent in commercial greenhouses compared to chemical pesticides, *H. variegata* to be assessed later. Results of both studies will be discussed considering the potential of the two predators for development as biocontrol agents of greenhouse pests.

A COMPARISON OF POPULATION GROWTH PARAMETERS OF
LADY BEETLE, *HIPPODAMIA VARIEGATA* GOEZE (COL:
COCCINELLIDAE) AND THEIR PREY,
APHIS GOSSYPYII GLOVER (HOM: APHIDIDAE) UNDER
LABORATORY CONDITIONS.

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Adult lady beetles *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) and melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphidae) were collected from cucumber fields in Rasht area and were reared in a growth chamber at a temperature at $26 \pm 2^\circ\text{C}$, RH $65 \pm 5\%$ and a photoperiod of 16L:8D. Ten mated pairs of two-day-old predators were separately transferred into a plastic container (8×10×12 cm) that were covered with an organdy net. By using daily fertility results, a demographic life table was constructed. Intrinsic rate of increase (r_m) and net reproductive rate (R_0) of *H. variegata* were 0.254 and 387.9, respectively. The mean generation time (T) of the lady beetle was 23.46 days and the population was doubled (DT) in 2.73 days. The population of *H. variegata* was able to multiply 5.9 times per week (r_w). In order to determine r_m and R_0 of the melon aphid, 20 wingless adults were put separately into a leaf cage (10×60 mm) on cucumber leaves. R_0 and r_m values of the aphid were 49.256 and 0.471, respectively. The mean generation time (T) was 8.27 days and the population of aphid was doubled (DT) within 1.47 days. Female aphids were able to multiply 27.03 times per week (r_w). Adult longevity was 13.8 ± 1.09 days and mean number of offspring produced per female was 49.05 ± 2.32 and mean number of females per female per day (m_x) was 3.22 ± 0.59 .

PESTICIDE-FREE PRODUCTION OF GREENHOUSE VEGETABLE CROPS USING BENEFICIAL INSECTS AND BANKER PLANTS

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Greenhouse production of vegetable crops in the USA and throughout much of the world has relied heavily on chemical insecticides to control arthropod pests in the past. Due to the constant application of certain insecticides under protected agriculture conditions, it is feared that insect resistance has occurred, especially with *Bemisi tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotype 'Q'. Consumer food safety concerns over chemical residues have furthered the advancement of alternative pest control measures. Arthropod pests are controlled solely with IPM and beneficial insects, including the banker plant inoculation method at the University of Florida Protected Agriculture Greenhouse Project (UFPAP), USA. Most common pests in vegetable crops produced in Florida include: two-spotted spider mites (TSSM), thrips, aphids, broadmites, and whitefly. Beneficial insects include: parasitic wasps for aphids and whitefly, predatory mites for TSSM and broadmites, and generalist predators such as *Orius* sp. (Hemiptera: Anthocoridae) for control of many pests including thrips. The use of banker plants in recent years has led to new research where the parasitic wasp *Encarsia sophia* (Girault & Dodd) (Hymenoptera: Aphelinidae) is reared on hosting papaya whitefly, *Trialeurodes variabilis* (Quaintance) (Hemiptera: Aleyrodidae), providing control over *B. tabaci* in greenhouse tomato, pepper and melon. A current overview of successful IPM methodology used in the UFPAP greenhouse is an important part of teaching growers on how to use the system. Using IPM techniques, including banker plants, will reduce costs to the grower for purchasing un-needed beneficials, increase potential for new industry to raise banker plants, reduce and potentially eliminate chemical applications increasing worker safety, avoid re-entry delays and ultimately produce a pesticide-free commodity.

EFFECTS OF MODERN INSECTICIDES IN THE VERY SENSITIVE PARASITOID *PSYTTALIA CONCOLOR* (SZÈP.) (BRACONIDAE) AND THREE SELECTED NATURAL ENEMIES OF IMPORTANCE IN HORTICULTURAL CROPS: THE PARASITOIDS *APHIDIUS ERVI* (HALIDAY) (APHIDIIDAE) AND *ERETMOCERUS MUNDUS* (MERCET) (APHELINIDAE) AND THE PREDATOR *CHRYSOPELTA CARNEA* (STEPHENS) (CHRYSOPIIDAE)

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The success of biological control agents in preventing pest outbreaks in protected crops has led us to be very aware of the need to apply selective pesticides because even though their use as a sole pest control strategy is desirable, it is insufficient in most cropping systems. Side effects of six modern pesticides with different modes of action were tested at the maximum field recommended rate in Spain (mg/l of a.i.): Spiromesifen (Oberon®; 137.4), Methoxifenozone (Runner®; 90.8), Flubendiamine (Fenos®; 60), Abamectine (Vertimec®; 18), Enamectine (Affirm®; 14.25) and Spinosad (Spintor®; 120). Deltamethrin was used as positive standard (Decis®, 50). Under laboratory conditions, the residual or ingestion toxicity was tested on adults or pupae of the very sensitive parasitoid *Psyttalia concolor* (Szèpligetti) (Hymenoptera: Braconidae) and 3 natural enemies of importance in horticultural crops in Spain: *Aphidius ervi* (Haliday) (Hemiptera: Aphidiidae), *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). Methoxifenozone, Spiromesifen and Flubendiamine were generally the safest for the enemies, and abamectin and deltamethrin the most harmful. When *A. ervi* adults fed continuously the insecticides at 21°C, abamectin and emamectin caused 100% mortality at 72h, deltamethrin 50%, and the rest of insecticides were harmless. The toxic effects were faster and more severe with the increase of temperature (24°C). Pupae of *E. mundus* were tolerant to every insecticide but spinosad. Fresh residues or ingestion of deltamethrin and spinosad negatively affected *P. concolor* adults. *C. carnea* adults were very sensitive to fresh residues of deltamethrin.

SESSION 11

BIOLOGICAL CONTROL OF PHYTOPHAGOUS MITES - THEORY AND PRACTICE

THE PREDATORY MITE SPECIES COMPLEX IN AUSTRALIAN VINEYARDS: PHYTOSEIIDAE, BDELLIDAE, AND ANYSTIDAE, INCLUDING SEASONAL POPULATION DYNAMICS OF ANYSTIDAE IN NORTH-EAST VICTORIA AND REDUCING THE IMPACT OF PESTICIDES ON BIOLOGICAL CONTROL IN VINEYARDS: MORTALITY AND FECUNDITY EFFECTS OF PESTICIDES ON AN INDICATOR SPECIES, THE PREDATORY MITE *EUSEIUS VICTORIENSIS* (ACARI: PHYTOSEIIDAE)

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Predatory mite species were surveyed in vineyards in South Australia and Victoria, providing new information on Phytoseiidae, Bdellidae and Anystidae species present, Phytoseiidae species feeding on the grape rust mite, *Calepitrimerus vitis* (Nalepa) (Acari: Eriophyidae), and limited population dynamics of *Anystis baccarum* (L.) (Acari: Anystidae), for one year in North-East Victoria. Mortality and fecundity effects of 33 pesticides, two surfactants, and various rates of wettable sulphur registered for use in viticulture, were studied on two predatory mite indicator species *Euseius victoriensis* (Womersley) and *Galendromus occidentalis* (Nesbitt) (Acari: Phytoseiidae) under 'worst-case scenario' direct overspray exposure in the laboratory. Tests were carried out on 8-48 h old juveniles, on detached soybean or French bean leaves, and standardized to deliver a pesticide dose comparable to commercial application of the highest registered label rate per 1000 L/ha spray volume. Results delineate pesticides per low, moderate, and high toxicity IOBC ratings, and conclude that mancozeb, carbendazim, benomyl, wettable sulphur (≥ 300 -400 g/100 L), and insecticides emamectin benzoate, spinosad, and to a lesser degree the fungicide pyrimethanil, are highly toxic to predatory mites. The usefulness of *E. victoriensis* as an indicator species for pesticide effects on vineyard bio-control was reviewed in the context of pesticide effects on other key biological control providers.

BIOLOGY AND FEEDING POTENTIAL OF THE PREDATORY MITE, *AMBLYSEIUS LONGISPINOSUS* (EVANS) ON EUROPEAN RED SPIDER MITE, *PANONYCHUS ULMI* (KOCH)- A SPIDER MITE INJURIOUS TO APPLE.

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Apple is one of the most important fruit crops grown in North Western Himalaya of India. One of the major factors which limits its successful cultivation is the attack of mite pests which reduces quality and yield. Among mites, the European red mite, *Panonychus ulmi* (Koch) (Acari: Tetranychidae) causes maximum losses to apple. Mite outbreak in apple orchard is mainly due to indiscriminate use of pesticides which cause suppression of natural enemies and leads to development of resistant pest strains. Of various biocontrol agents, predatory mites of the family Phytoseiidae (Acari: Mesostigmata) have been recorded since the beginning of 20th century on phytophagous mites, among which *Amblyseius longispinosus* (Evans) is one of the most important predators. *Amblyseius longispinosus* was tested for the control of tetranychid mites on various crops other than apple. The present studies were conducted under laboratory conditions (12 - 28.5°C and 70-98%). The data on biological parameters revealed that the total life period for male and female was completed in 27.06 and 27.55 days respectively. The male to female ratio was 1:4.55. The adult female laid 34.84 eggs during its oviposition period of 17.72 days. The data on feeding potential showed that protonymph, deutonymph and adult of predator had consumed 5.37, 5.97 and 73.7 nymphs and 5.34, 5.79 and 84.86 adults when fed exclusively on nymphs and adults of *P. ulmi*, respectively. However, they had consumed more number of nymphs and adults as compared to eggs when fed on mixed developmental stages of *P. ulmi*. In all cases larva of the predator did not feed.

RELEASE OF *NEOSEIULUS CALIFORNICUS* ON PEPPER TRANSPLANTS TO PROTECT GREENHOUSE-GROWN CROPS FROM EARLY BROAD MITE (*POLYPHAGOTARSONEMUS LATUS*) INFESTATIONS

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Broad mites, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae), rapidly damage pepper, *Capsicum annuum* L. (Solanaceae), crops and reduce production in greenhouses in warm climate regions when the minute pest infests at early plant developmental stages. An initial study was set up to evaluate biological control strategies during seedling development. Timing of release of *Neoseiulus californicus* McGregor (Acari: Phytoseiidae), a commercially reared predatory mite, was investigated in scenarios of pest and predator presence to simulate preventive and curative control strategies. Undamaged seedlings were produced when two predators per seedling were released preventively. Curative control strategies were not effective for producing undamaged or broad mite-free transplants. Further studies were carried to evaluate the effectiveness of *N. californicus* on pepper seedlings that host a few broad mites and are transplanted into the production greenhouse. *Neoseiulus californicus* released at different times and densities, and conventional control methods (micronized-sulfur sprays) were evaluated for pest control, plant damage, and fruit yield. Yields of red fruits from plants with four predators per plant released at any of the release times (a week before, at, or four days after transplanting) or with sulfur sprays were not significantly different from an uninfested control treatment. Releases of two predatory mites per plant led to yields similar to the uninfested control only when introductions were a week before transplanting, while later introductions led to serious plant damage and significantly lower yields. Early broad mite infestations in pepper crops grown in greenhouses might be effectively controlled with *N. californicus* released at early seedling developmental stages in the nursery and at transplanting in the fruit production greenhouse.

DOES IMIDACLOPRID INDIRECTLY AFFECT BEHAVIOR AND PERFORMANCE OF PREDATORS OF SPIDER MITES ON LANDSCAPE PLANTS?

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Imidacloprid was the first neonicotinoid insecticide to be introduced to the landscape plant market. It is still one of the most common materials applied in landscapes owing to its broad range of activity against many insect pests. Recently, several studies documented elevated populations of spider mites and increased levels of mite injury on plants treated with imidacloprid. One explanation for these results is that spider mites exposed to plants treated with imidacloprid ingest the insecticide and become toxic to their predators. We tested this hypothesis by rearing spider mites under two insecticide regimes. One group of boxwoods and elms were treated with soil drenches of imidacloprid. Another group of plants were not treated with imidacloprid. Plants were then infested with spider mites. After several weeks of exposure, mites from treated and untreated plants were collected and offered to lacewing larvae and lady beetle adults. We recorded feeding rates, mobility, and survival of lacewings and lady beetles that consumed mites from treated and untreated plants. Lacewing larvae and lady beetle adults experienced reduced mobility, reduced rates of feeding, and reduced survival when fed spider mites from

plants treated with imidacloprid compared to prey from untreated plants. Indirect exposure to imidacloprid through toxic prey may reduce the ability of predators to suppress spider mites on treated plants. This loss of top-down regulation may contribute to outbreaks of spider mites on plants treated with imidacloprid.

A SURVEY OF PREDACIOUS MITES (ACARI: PHYTOSEIIDAE) IN NORTH COAST CALIFORNIA VINEYARDS

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Native and introduced predacious mites in the family Phytoseiidae are important biological control agents of spider mites and thrips in California vineyards. The types of insecticides used in vineyards have dramatically changed in the last ten years from organophosphates and carbamates to a number of different chemical classes, many of which are more selective. This might have impacted phytoseiids diversity and abundance. Exotic pest mites and thrips have become established many times throughout California history and continue to be introduced. A better understanding of the predacious mites present in vineyards may aid in managing new pests. We undertook a three-year survey of phytoseiids in 21 vineyards in four North Coast counties of the winegrape growing region of California. Each site was monitored monthly from May to November. The most abundant predacious mite found was *Typhlodromus pyri* Scheuten followed by *Euseius quetzali* McMurtry and *Galendromus occidentalis* Nesbitt. On average two species of phytoseiids were found per site per date with a range of 1 to 7 species. Approximately the same phytoseiid species composition was found at each site through the season and through the three year surveyed. A total of 15 species were collected from grapevines. Two species had never been reported from California: *Amblyseius andersoni* Chant and *Graminaseius graminus* (Chant).

SESSION 12

ATTRIBUTES OF EXOTIC BIOLOGICAL CONTROL AGENTS: THE GOOD AND THE BAD

RETROSPECTIVE NATURAL AND NOVEL RANGE RESEARCH ON THE BRACONID PARASITOID *MICROCTONUS AETHIOPOIDES* LOAN

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The Moroccan biotype of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) was released in New Zealand in 1982 as a biological control agent for *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae). Since then it has been found to attack 19 non-target weevil species in the field including 14 native species. Natural and novel host-range research has often been useful to inform biosafety concerns for proposed biological control agents. In this study, this has been carried out retrospectively, to determine whether natural and novel range research would have assisted in predicting the host range that has been recorded in New Zealand. Sampling has been carried out in the natural range, Morocco, and in SE Australia, where it has also been introduced for biological control. Results to date have revealed only one non-target host species in SE Australia.

BIOLOGICAL CONTROL: ENVIRONMENTALLY FRIENDLY OR RISKY BUSINESS? A CASE STUDY WITH THE PREDATORY MIRID *NESIDIOCORIS TENUIS*

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Biological control is often regarded as a 'green' approach to pest management. However, as this approach is not without risk, legislation has been implemented in many parts of the world to regulate the introduction of non-native biological control agents. *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) is a polyphagous mirid currently used to great effect for the control of glasshouse pests in Mediterranean regions to which it is indigenous. As a result of this success, licenses for the use of these insects are being sought in temperate regions such as the UK. In accordance with UK legislation, such licenses require a thorough pre-release risk assessment, covering issues such as establishment potential outside of glasshouse environments

and impact on non-target prey. As temperature is the most important factor affecting the establishment of alien species in the UK, this work primarily investigates the thermal tolerances of *N. tenuis* through a series of laboratory and field experiments. Results to date indicate a very low level of cold tolerance, with 100% mortality after 3-4 weeks of winter exposure. Together with a developmental threshold of 14°C, an LTime₅₀ at 5°C of only 9 days and an inability to diapause, these data indicate that *N. tenuis* is unlikely to establish in the UK and would therefore constitute a 'safe' biological control agent.

SIGNIFICANCE OF PARASITISM AND PREDATION TO MORTALITY OF *PHYLLOCNISTIS CITRELLA* POPULATIONS IN SPAIN

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The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a pest native of Southeast Asia which posed a serious threat to the citrus industry of the Mediterranean region upon its introduction in 1993 and its subsequent spread. Immediately after its introduction, a classical biological control program comprising the introduction and release of six exotic parasitoid species was implemented in Spain. In 2006, data on the incidence of the pest were collected and the impact of both exotic and indigenous natural enemies on *P. citrella* populations was measured following the same protocols used at the early years of the follow up of the biological control program (1997-1999). Results showed that more than 99.0% of the parasitoid specimens collected in 2006 corresponded to the exotic eulophid *Citrostichus phyllocnistoides* (Narayanan) (Hymenoptera: Eulophidae). Other parasitoids, either indigenous or introduced, accounted for less than 0.4% of the total catches. From 1999 to 2006, the incidence of the citrus leafminer decreased (3.2-5.1 vs. 1.8-2.4 mines per leaf for 1997-9 and 2006, respectively). Both parasitism and feeding punctures significantly increased (from 8.6 to 16.4%, and from 9.4 to 19.3%, respectively), but both predation and overall impact of natural enemies did not significantly change (19.5 and 16.5%, and 36.8 and 48.4%, respectively). These results allow for a post-release evaluation of the biological control program against the citrus leafminer carried out in Spain.

DEVELOPMENT OF *MICROCTONUS AETHIOPOIDES* LOAN THROUGH MULTIPLE GENERATIONS OF NOVEL WEEVIL HOSTS IN THE LABORATORY

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Host range testing of entomophagous parasitoids is a key step in the evaluation of biological control agents prior to any release in a classical biological control programme. However, while the value of these tests provides some confidence in assessing parasitoid host specificity, they rarely span more than a single generation and hence provide few insights to novel host acceptance over multiple generations. Pre-release studies on non-target hosts spanning successive parasitoid generations provide the opportunity to evaluate temporal changes in behavioural and physiological acceptability, parasitoid fecundity and longevity compared to the target species. Retrospective post-release studies may help determine if field non-target parasitism is due to the development of self-sustaining parasitoid populations. This approach was examined using two biotypes of the endoparasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), introduced to New Zealand from Morocco and Ireland, for biological control of *Sitona discoideus* and *S. lepidus* (Coleoptera: Curculionidae), respectively. Laboratory studies on non-target acceptance by the parasitoids on two New Zealand native weevils, *Irenimus aequalis*, and *Nicaeana cervina*; one introduced weed biocontrol agent, *Rhinocyllus conicus*; and an introduced pest weevil, *Listronotus bonariensis* (Coleoptera: Curculionidae) showed that both parasitoids could be successfully reared through three generations on these novel hosts, as well as demonstrated comparative differences in acceptance and parasitoid development rates. However, it was found that weevil phenology and physiology had an effect on the ability to sustain parasitoid development across generations, a factor that could have implications for potential non-target impacts in the field.

TEN YEARS OF REGULATED RELEASES OF BIOLOGICAL CONTROL AGENTS IN NEW ZEALAND

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Since July 1998, 15 biological control agents, 12 for weed and three for insect control have been released in New Zealand. These releases were approved under the Hazardous Substances and New Organisms (HSNO) Act that requires an environmental risk assessment to be undertaken. As the decision maker under the act the Environmental Risk Management Authority (ERMA New Zealand) has encountered areas of uncertainty in assessing applications to release these insects. The major areas of uncertainty have been the displacement of native species (adverse non-target effects on native or valued organisms), compatibility of existing biological control agents or agents within multiple releases, and potential for differential host range of biological control agent biotypes. The latter has led to applications for release with controls limiting releases to specific geographical biotypes. Examples of releases include *Cleopus japonicus* Wingelmuller (Coleoptera: Curculionidae) for *Buddleja davidii* (Franchet) (Buddlejaceae), *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) for *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae), and *Ceratapion onopordi* (Kirby) (Coleoptera:

Apionidae) for *Cirsium arvense* (L.) (Asteraceae). To date ERMA New Zealand has not declined a biological control agent for release however, potential applicants are involved in extensive pre-application discussion before an application is submitted. ERMA New Zealand has clearly signalled that in the future, data on the interaction between multiple biological control agents will be required for an application to release to be successful.

METEORUS PULCHRICORNIS: AN EXOTIC POLYPHAGOUS PARASITOID DISRUPTING CLASSICAL BIOLOGICAL CONTROL OF *HELICOVERPA ARMIGERA* IN NEW ZEALAND: YES OR NO?

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Meteorus pulchricornis (Wesmael) (Hymenoptera; Braconidae) is an exotic, polyphagous, self-introduced parasitoid which has been recorded parasitizing >30 native and exotic larvae of lepidopteran species in urban and agro-ecosystems since its arrival in New Zealand in 1996. Research in outdoor vegetables shows that in a multi-cropping environment where there are numerous alternative lepidopteran host species, *M. pulchricornis* is displacing *Cotesia kazak* (Telenga) (Hymenoptera: Braconidae), the important introduced specialist parasitoid of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). However, overall levels of parasitism in processing tomato crops, where biological control of larvae of *H. armigera* is an important component of an IPM programme, is not greatly affected, remaining at about 75% parasitism. In a mono-cropping environment, where *H. armigera* is the only commonly available host, *C. kazak* remains the dominant parasitoid species, with about 90% of parasitised larvae producing *C. kazak*. We report laboratory studies on the developmental biology of the two introduced specialist larval parasitoids of *H. armigera*, *C. kazak* and *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), compared with *M. pulchricornis*, showing that *C. kazak* has a physiological advantage over the other two species, being able to parasitise smaller larvae and develop more quickly. These studies also include inter-specific competition, pair-wise assays among the three parasitoid species, including 0, 24 and 48 hour delays in parasitisation between two species. Our results are discussed and we also outline the overall effects that the exotic *M. pulchricornis* is having on the biological control of *H. armigera* and on the wider New Zealand fauna.

SESSION 13

INDUCIBLE PLANT RESPONSES AND ITS IMPACT ON BIOLOGICAL CONTROL OF PLANT PESTS

SILICON AND INDUCED PLANT DEFENCES: ENHANCING NATURAL ENEMY ATTRACTION TO PEST-INFESTED PLANTS

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Given the evidence for a role of silicon in constitutive and induced plant defences, it is surprising that virtually all published work has focused on two-trophic level studies (ie plants and arthropods or plants and pathogens). There is good reason for suspecting that silicon may have yet-to-be-recognized significance in induced plant defences involving attraction of natural enemies of arthropod pests. Silicon-supplemented plants translocate silicic acid throughout their tissues and, when attacked, produce systemic stress signals such as salicylic acid and jasmonic acid that are key to induced plant defences. Silicon has been postulated to play two important roles in plant chemical defence: (1) acting as a modulator of metabolic signalling events at the cellular level and (2) the generation of metabolic signals that give a systemic response at the whole plant level. Jasmonic acid is particularly important because this compound can trigger production of herbivore-induced plant volatiles (HIPVs) by the attacked plant. These chemical signals 'recruit' predators and parasitoids. This paper will present results from current studies of the effects of soil-applied silicon on natural enemies in a model system comprising cucumber, *Cucumis sativus* L. (Cucurbitaceae) and a range of arthropod pests and natural enemies. Findings will be presented on the attraction and performance of parasitoids and predators in Y-tube olfactometer bioassays and free choice experiments comparing infested and uninfested plants with and without potassium silicate. Results will be discussed in relation to the applied conclusions for enhancement of biological control as well as the implications for chemical ecology of food webs.

ZOOPHYTOPHAGOUS BUGS INDUCE WOUND RESPONSE

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In tomato plants, wounding and damage by insects induces the wound response. This causes defense molecules like proteinase inhibitors to be produced. Important molecules in the signalling pathway of this response are systemin,

jasmonic acid and ethylene. Certain frequently used natural enemies in vegetable greenhouses are facultatively phytophagous, which means that these predators not only feed on arthropod prey but also on the plants. Moreover they deposit their eggs inside the plant tissue. As a result, these natural enemies could switch on the plant defense. By means of RT-PCR the expression level of certain genes involved in the wound response (proteinase inhibitor 1, prosystemin and allene oxide synthase) was examined in tomato colonized by the predatory bugs *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae). Both insects caused a very strong upregulation of the proteinase inhibitor 1 gene. The expression level reached a peak around 24 hours after the insects were placed on the plants. Moreover, the upregulation was stronger during infestation with the more phytophagous *M. pygmaeus*. The other investigated genes were also upregulated, but the maximum expression level occurred earlier and upregulation was lower than for the proteinase inhibitor 1 gene.

INDUCTION OF PLANT IMMUNITY TO ARTHROPOD HERBIVORES AND PATHOGENS USING BIOTIC AND ABIOTIC ELICITORS

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Plants, during their life cycle, protect themselves against the attack of arthropod herbivores and pathogens with various resistance mechanisms. The jasmonate and salicylate signaling pathways are two inducible defense responses that can protect plants against these multiple pests by the production and synthesis of a number of phytochemicals and defensive proteins such as proteinase inhibitor proteins, polyphenol oxidases and steroid glycoalkaloids as well as pathogenesis-related proteins (PR-proteins) and phytoalexins that target physiological processes in the attacking organisms. Secondary metabolites produced by plants in response to induction by biotic or abiotic elicitors also include volatile organic compounds that attract natural enemies of insect herbivores. Research efforts in the area of inducible plant resistance is now being developed concerning the use of induced plant genes for insect herbivory and pathogen resistance and the potential of using induced resistance (IR) or systemic acquired resistance (SAR) as environmentally safe methods of insect and disease pest control, respectively. This paper views this research as a body to fully utilize the information in formulating future development of plant protection products and farming practices that can assist growers in controlling arthropod herbivores and pathogen attack on high cash value plant crops. The integration of induced resistance and semiochemical approaches with other methods of pest management would be accepted as compatible with integrated pest management system in conventional agriculture as well as with organic farming practices.

ENHANCING THE ATTRACTIVENESS OF MAIZE ROOTS FOR ENTOMOPATHOGENIC NEMATODES

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When attacked by insects, plants emit volatiles that can serve as cues for predators and parasitoids in search of herbivorous prey. It has been proposed that the emissions of these volatile signals can be manipulated to improve crop protection. We demonstrated the full potential of this strategy by restoring a specific belowground signal emitted by insect damaged maize roots. This signal, the sesquiterpene (E)-BETA-caryophyllene, is highly attractive to the entomopathogenic nematode *Heterorhabditis megidis* Poinar, Jackson & Klein (Heterorhabditidae: Rhabditida) and is emitted by ancestral maize and European lines, but not by most American varieties. The loss of the signal was previously found to strongly reduce the effectiveness of the nematode as a control agent of the larvae of the western corn rootworm (WCR), a ferocious root pest. To restore nematode attraction, a non-producing maize line was transformed, resulting in constitutive emissions of (E)-BETA-caryophyllene. In WCR-infested field plots in which we released nematodes, transformed lines received significantly less WCR damaged and had fewer adult beetles emerge than isogenic lines. These differences were not observed in plots without released nematodes. We have also been successful at selecting a strain of the highly infective nematode *H. bacteriophora* to better respond to (E)-BETA-caryophyllene. Field tests with this new strain show that by combining plants that emit the appropriate signal and nematodes that respond well to this signal, biological control can be just as effective as conventional WCR control strategies.

USE OF KEY COMPOUNDS IN DISCRIMINATION OF DIFFERENT PLANT SPECIES BY ASSOCIATIVE LEARNING IN THE EGG-LARVAL PARASITOID, *ASCOGASTER RETICULATA* WATANABE (HYMENOPTERA: BRACONIDAE)

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In order to locate a host insect, a parasitoid has to find a suitable host habitat with the help of plant chemicals. Once a female wasp finds a host on some plant, she begins to search for the plant-related chemicals that enable efficient host finding. The learning behaviour of the egg-larval parasitoid *Ascogaster reticulata* (Watanabe) (Hymenoptera: Braconidae) was investigated using five chemicals identified from tea leaves. Learning efficiency of geraniol and linalool was higher than the other 3 components, (Z)-3-hexenol, methyl salicylate and benzyl alcohol. Discrimination between geraniol and linalool was clarified when the number of conditionings increased. Furthermore, *A. reticulata* females prefer successive oviposition

experience and newly-learned information. Two-choice bioassays using mixtures of the tea leaf components suggested that geraniol and linalool play a key role in discrimination by ovipositing females. However, *A. reticulata* females could also discriminate the minor components in the mixture. They may use several key components in discrimination of plant species as well as other hymenopteran species. Preference of successive oviposition experience is suitable for female's host-searching behavior in the field.

OLFACTORY RESPONSES OF THE PARASITOID *APHIDIUS COLEMANI* TO THE COMPANION PLANT, *BORAGO OFFICINALIS*

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In a strawberry greenhouse, aphid parasitoids are often observed to gather around borages, *Borago officinalis* L. (Boraginaceae), used as banker plants (Nemoto & Omori, 2007). We investigated the attraction of *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) to *B. officinalis* as a model aphid parasitoid. *A. colemani*, an aphid generalist parasitoid, shows a preference for the host-plant volatiles on which their host aphids are reared based on their experience after hatching from mummies. However, the preference of *A. colemani* for particular plants has not been reported. *A. colemani* colonies reared on two host plant-aphid systems, the Japanese radish-green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphidae), and the wheat-oat bird cherry aphid *Rhopalosiphon padi* L. (Hemiptera: Aphidae), were used in these experiments. A 4-arm olfactometer was used to test the preference of female *A. colemani* for several plants (infested Japanese radish; infested wheat; uninfested, infested and mechanically-damaged borage). When infested Japanese radish and wheat were used, both female groups showed preference only for the plant on which their hosts were reared. On the other hand, female parasitoids were attracted to infested borage regardless of the host plant-aphid system. These results suggest that aphid-infested borage attracts *A. colemani* by a mechanism different from that based on their experiences after hatching from mummies.

ANTIXEOSIS OF SOME MELON (*CUCUMIS MELO*) ECOTYPES OF IRAN TO TWO-SPOTTED SPIDER MITE (*TETRANYCHUS URTICAE*) IN GREENHOUSES

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The two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) is one of the most important pests of cucurbits, that can infests more than 200 species. In order to find resistance sources in melon, the antixenosis of 36 ecotypes collected from different parts of Iran, was evaluated in greenhouse. Ten seeds of each ecotype were cultivated in pots (one seed per pot). At six leaf stage, one leaf (about 4 cm²) from each plant was placed around a circle with a diameter of 100 cm. Experimental design was an alfa-lattice (0-1) with 9 ecotypes in each sub-block and three replications. Mean comparisons were performed using Duncan's multiple range test. In order to determine the antixenosis, ten adult females were released at the center of circle for each leaf. The number of mites feeding on each leaf was recorded after 24 and 48 hours. Statistical analysis showed significant differences between genotypes and indicated antixenosis resistance ($p < 0.05$). Maximum number of mites were observed in Garmsari (17.73 ± 0.263) and Zard (15.22 ± 0.515) and the minimums were observed in Dastanbo Aran-Bidgol (2.58 ± 0.288) and Samsuri Ghermez (3.02 ± 0.066). Therefore, the above mentioned ecotypes are supposed to be typically susceptible and resistance to *T.urticae* respectively.

SYNTHETIC HERBIVORE INDUCED PLANT VOLATILES- A TOOL FOR ENHANCING CONSERVATION BIOLOGICAL CONTROL OF CROP PESTS

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In response to arthropod damage, plants have evolved to emit volatile signalling compounds also known as herbivore induced plant volatiles (HIPV's). These attract natural enemies of the attacking herbivore. Several HIPV compounds have been identified from different plant species. Previous research has demonstrated that synthetic versions of common HIPV's, when deployed directly into the crop lead to attraction of natural enemies, potentially improving efficacy of conservation biological control (CBC). This project is aiming to enhance CBC by exploring this novel approach and understanding whether natural enemies are attracted to applied HIPV's or to volatiles emitted by plants that have been exposed to synthetic HIPV's. Six synthetic HIPV's (methyl salicylate (MeSA), methyl anthranilate (MeA), methyl jasmonate (MeJA), benzaldehyde (Be), cis-3-hexenyl acetate (HA), cis-hexen-1-ol (He)) at three concentrations (0.5, 1.0 and 2.0%) were applied to winegrapes, broccoli and sweet corn to test their efficacy. Results from these experiments revealed significant differences between treatments and thus demonstrating attraction of parasitoid families to HIPV's. Trichogrammatidae responded to Be (1.0%) and MeA (0.5 and 1.0%), Encyrtidae to MeA (0.5%) and Scelionidae to MeSA (2.0%). Arthropods were monitored at day 1, 4, 8 and 11 after HIPV application and only at day 4 were greater numbers of parasitoids were found, indicating that

synthetic HIPV's may signal plants to produce their own volatiles. Enhanced retention of parasitoids may be achieved through combining HIPV's with rewards of floral food sources. Findings will be discussed in relation to the potential use of synthetic HIPV's for integrated pest management strategies.

SESSION 14

FOOD WEB INTERACTIONS AND IMPACT ON BIOLOGICAL CONTROL

RICHNESS AND ABUNDANCE OF ARTHROPODS ON WEEDS OF MAIZE CROPS AT HIGHLANDS REGION OF CHIAPAS, MEXICO

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The richness and abundance of arthropods that live in 14 maize fields were studied in Yaluma Villahermosa town, Comitan municipality, Chiapas State, Mexico. The arthropods and weed communities were sampled every month for a year using a whole bag sampling method (WPBS). Arthropods were grouped according to their feeding conduct: phytophagous, predators, pollinators, parasitoids and decomposers. A Pearson correlation statistical test was performed to determine if there is a relation ship between morpho-species and weeds. The result was negative. In 18 weeds were 616 Arthropods, belonging to 11 orders, 67 families and 95 morpho-species. The greatest diversity was collected in *Tithonia tubaeformis* (Jacq.) Cass., *Bidens odorata* Cav. and *Dyssodia tagetiflora* Lagasca (Asteraceae). The Arthropoda orders with the highest number of families are: Hemiptera, Hymenoptera and Diptera; while Coleoptera has the highest number of morpho-species (30) and individuals (425). Phytophagous arthropods were the most abundant, followed by predators and then parasitoids. This information could be useful for the future management of weeds for insect manipulation in maize agroecosystems at Highlands region of Chiapas.

DYNAMICS OF THE FOLIOPHAGOUS TORTRICIDS COMPLEX (LEP., TORTRICIDAE) IN A COMMERCIAL APPLE-TREE ORCHARD CONVERTED TO BIOLOGICAL CONTROL OF PESTS

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In the north-eastern Romania over 20 species of phytophagous torticids are recorded in the insecticide-free apple-tree orchards. Their number is greatly diminished in the commercial, conventionally exploited orchards, where some of them became main pests, e.g. the summer fruit tortrix moth, *Adoxophyes orana* Fisch. v. Rosl (Lepidoptera: Tortricidae). In this paper we present aspects regarding the dynamics of the phytophagous torticids from a 10 ha plot of an intensive apple-tree orchard that was converted to an ecological production since 2006, when

synthetic insecticides were eliminated. The main strategy for controlling the lepidopteran pests was using the oophagous wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae), with 6-8 releases per vegetative season, consisting in 600.000-800.000 individuals/ha. After three years with no chemical control, 9 phytophagous tortricids species were recorded in the ecological plot, including *Archips rosana* L., *Ptycholoma lecheana* L., *Pandemis heparana* Den. & Schiff., *Pandemis cerasana* Hb., *Aphelia viburniana* Fabr., *Adoxophyes orana* (Fischer von Rösslerstamm), *Hedya nubiferana* Haw., *Rhopobota naevana* Hüb. and *Spilonota ocellana* Den. & Schiff.; at the same time, only 3 species, *A. rosana*, *P. heparana*, *A. orana*) were recorded in the chemical plot.

POSITIVE PLANT-APHID INTERACTIONS: SYNERGY BETWEEN INDUCED DEFENSE AND APPARENT COMPETITION

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Aphids have strong direct and indirect effects on many members of natural and agricultural food webs. Aphid density has direct negative effects on plants by phloem feeding. However, through indirect interactions, aphids have the potential to return a net benefit to plants. Aphids induce changes in plant chemistry which reduce plant quality for other, more damaging herbivores. Further, aphid density can increase the abundance of natural enemies with negative consequences for other herbivores. We predict that cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphidae), will promote indirect effects on the diverse arthropod community of cotton, *Gossypium hirsutum* L. (Malvaceae), via aphid-induced changes in plant chemistry and aphid-induced changes in the abundance and behavior of predators. We further predict that the net effect of cotton aphids on cotton yield will be positive. In laboratory experiments, we demonstrate that aphids increase the density of pigment glands on cotton leaves. These glands contain gossypol which is toxic to caterpillars. As such, oviposition by beet armyworm moths, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), and caterpillar growth is reduced when aphids are present. Cotton aphids also have negative indirect effects on caterpillars via shared natural enemies. Generalist predators spend more time on plants with aphids than those without. This translates into significantly greater predation of caterpillars. Results of a factorial field cage experiment indicate that reduced caterpillar load on plants and increased predator abundance results in synergy between induced defenses and apparent competition. This research describes a novel set of interactions that alter the strength of trophic cascades thus promoting a positive plant-herbivore interaction.

MANAGING PESTIFEROUS AQUATIC MIDGE EMERGENCES FROM STORM WATER RETENTION PONDS

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Residents living adjacent to storm water retention ponds in the community of Saint Lucie West (Florida, USA) complained about the emergence of large swarms of pestiferous aquatic midges. Aquatic midges are mosquito-like Diptera belonging to the families Chironomidae and Chaoboridae. Chironomidae are commonly referred to as “Blind Mosquitoes” and Chaoboridae are commonly referred to as “Phantom Predatory Midges.” Midge eggs, larva, and pupa live in storm water retention ponds. Larvae are often referred to as wrigglers. These insects do not bite, suck blood, or carry diseases; however, they emerge from ponds in large numbers, primarily in the warm summer months. Because these insects are difficult to control, the Saint Lucie West Services District contacted the University of Florida - Saint Lucie County Cooperative Extension and requested help to devise and implement an aquatic midge integrated pest management (IPM) plan. The IPM plan that was adopted by the district featured the use of insect growth regulators, insectivorous fish, light traps, nutrient management and algae control strategies. The Pond Appeal Series was developed by the extension office to educate the Saint Lucie West Services District pond management crew and residents of the community about this IPM plan. All participants indicated they increased their understanding of pond management techniques and adopted practice changes that enabled them to better manage these ponds. This resulted in a reduction in pestiferous aquatic midges and improved quality of life.

WHICH ROLE DOES THE INDIGENOUS PARASITOID *BRACHYUFENS OSBORNI* (HYMENOPTERA: TRICHOGRAMMATIDAE) PLAY IN THE CLASSICAL BIOLOGICAL CONTROL PROGRAM AGAINST *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) IN FLORIDA?

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Diaprepes abbreviatus (L.) (Coleoptera: Curculionidae) is an exotic root weevil occurring in southern USA. It is a highly polyphagous species which can cause important economic losses. The lack of native egg parasitoids for this weevil in Florida triggered efforts to evaluate candidate egg parasitoids from the Caribbean Region. Four species have been released so far, *Aprostocetus vaquitarum* Wolcott (Hymenoptera: Eulophidae), *Haeckeliana sperata* Pinto (Hymenoptera:

Trichogrammatidae), *Fidiobia dominica* Evans & Peña (Hymenoptera: Platygasteridae) and *Quadrastichus haitiensis* (Gahan) (Hymenoptera: Eulophidae). Results have not been as satisfactory as expected and establishment has only been recorded in South Florida. *Brachyufens osborni* Dozier (Hymenoptera: Trichogrammatidae) is a weevil egg endoparasitoid native to Florida where it is presumed to occur on weevil egg masses of *D. abbreviatus*, as well as on indigenous weevils such as *Pachnaeus litus* (Germar) (Coleoptera: Curculionidae). Although this species has been reported parasitizing up to 81% of eggs of *P. litus*, its effect on *D. abbreviatus* remains unclear. The relationship between *B. osborni* and the introduced weevil egg parasitoids *A. vaquitarum*, *H. sperata* and *Q. haitiensis* has been studied in the laboratory. Results show that *B. osborni* is a highly effective parasitoid, both against *D. abbreviatus* and *P. litus*. However, it can not reproduce on *D. abbreviatus* and therefore its effect can not be identified in the field. Furthermore, when *B. osborni* occurs with the introduced parasitoids, the chances for the exotic species to complete their cycle decreases. These results show the important role that this native natural enemy may be playing in the biological control program developed against *D. abbreviatus*.

A DECISION-SUPPORT SYSTEM FOR THE SELECTION OF NON-TARGET SPECIES FOR ENVIRONMENTAL RISK ASSESSMENTS WITH ENTOMOPHAGOUS BIOLOGICAL CONTROL AGENTS

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The PRONTI (priority ranking of non-target invertebrates) decision-support system has been developed to improve the selection of non-target species for environmental risk assessment testing. This new, automated system should allow researchers to objectively assess as many outcomes from the introduction of a candidate biological control agent (BCA) as possible, thus improving the risk assessment process. The PRONTI system produces a prioritised list of non-target species where those at the top of the list are deemed to be the most at risk, and hence the most appropriate, candidates for risk assessment testing. The list is produced by ranking each non-target species according to five selection criteria: (1) potential hazard or benefit; (2) potential exposure; (3) environmental impact of a major change in the species' population following the introduction of the BCA; (4) anthropocentric value; and (5) testability of the species. The ranking process uses a specially designed database of information on the biology of each species found in the receiving ecosystem. "Scores" assigned to each piece of information are used in a model to produce the final PRONTI score: the larger the PRONTI score, the higher the species will appear on the prioritised list. Species may be prioritised because the BCA may be a direct or indirect hazard (e.g., from direct attack or loss of prey) or a direct or indirect benefit (e.g., to predators of the BCA or to prey of a species consumed by the BCA). The PRONTI lists should, therefore, provide guidance and support for decisions on which species should undergo risk assessment testing.

SESSION 15

PROGRESS AND PROSPECTS TO ASSESS PREDATION

PREDATORY POTENTIAL OF THE TIGER BEETLE *TETRACHA CAROLINA CAROLINA* ON COMMON TURFGRASS PESTS

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The tiger beetle *Tetracha carolina* (L.) (Coleoptera: Cicindellidae) occurs in turf and landscape beds in synchrony with common pests. *Tetracha carolina*'s abundance was influenced by turfgrass type and surrounding habitat. The common turfgrass pests *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), *Prosapia bicincta* (Say) (Hemiptera: Cercopidae), and *Popillia japonica* Newman (Coleoptera: Scrabaeidae) were suitable prey for *T. carolina* in laboratory studies. In petri dish trials, *T. carolina* and *Harpalus pennsylvanicus* DeGeer (Coleoptera: Carabidae) consumed similar numbers of *P. japonica* eggs. *Tetracha carolina* consumed more first instar *P. japonica* larvae than *H. pennsylvanicus* in petri dish assays however, when *T. carolina* and *H. pennsylvanicus* adults were confined with different densities of *P. japonica* (initially as eggs) for three weeks in soil in the laboratory, *H. pennsylvanicus* consumed a greater proportion of prey at all densities on day 2 and 1 week. Numbers consumed by week 3 were similar at the highest density, but still lower for *M. carolina* at lower densities. In field studies, however, where *P. japonica* and *T. carolina* or *H. pennsylvanicus* adults were released into plots simultaneously, there was no significant reduction in the subsequent grub population.

COMBINING ECOLOGICAL AND MOLECULAR METHODS TO INVESTIGATE PREDATION OF A LEPIDOPTERON PEST COMPLEX OF BRASSICA CROPS

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In southeast Queensland *Brassica* (Brassicaceae) crops are attacked by *Crociodolomia pavonana* F. (Lepidoptera: Crambidae) in late summer and autumn and by *Plutella xylostella* (L) (Lepidoptera: Plutellidae) from late autumn through spring. The impact of endemic predatory arthropods on each pest was investigated by their physical exclusion from experimental cohorts of *C. pavonana* and *P. xylostella* in May and August 2006, respectively. Throughout each study, randomly selected cabbage plants (n=20-40/ sample interval) were destructively sampled and predators on the soil surface were collected. All predators were stored (-18°C, ethanol (95%)) prior to

DNA analysis of gut contents to detect the remains of each insect. In May, the mean predator density was 1.8/ plant and 0.57 (95%CI = 0.40-0.74) of the *C. pavonana* cohort was lost to predation. Spiders (Araneae) accounted for 92% of all predators (Lycosidae (68%), Theridiidae (5%), Salticidae (5%), Clubionidae (3%), unidentified (11%). *Crociodomia pavonana* and *P. xylsotella* were detected in 23% and 5% of the collected Lycosidae (n=26), respectively. In August, the mean predator density was 2.1/ plant and 0.88 (95%CI=0.83-0.93) of the *P. xylsotella* cohort was lost to predation. Spiders accounted for 99% of all predators (Lycosidae (51%), Theridiidae (7%), Salticidae (2%), Clubionidae (5%), unidentified (32%). Remains of *P. xylsotella* were detected in Lycosidae (11%; n=95), Theridiidae (42%; n=12), Clubionidae (40%; n=10). *Plutella xylsotella* larvae are small and pupate on the host plant while *C. pavonana* are larger and pupate in the soil; these inter-specific differences probably explain the relative importance of foliar (Theridiidae and Clubionidae) and soil surface dwelling spiders (Lycosidae) as predators of each species.

cGMP-DEPENDENT ENHANCED PREDATION IN ADULT *CRYPTOLAEMUS MONTROUZIERI* MULSANT: A CLUE TO UNDERSTAND FORAGING BEHAVIOUR

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The predatory coccinellid, *Cryptolaemus montrouzieri* Mulsant (Coccinellidae: Coleoptera), feeds on vast range of mealybug species and other homopteran pests that are posing severe threat to many crops. Nevertheless, the causes for the relatively low rates of their field establishment and predation ability in the farmer's field have not been dealt with so far. A basis of natural polymorphism in foraging behavior was established in *Apis mellifera* L. (Hymenoptera: Apidae), *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) and *Caenorhabditis elegans* (Maupas) (Rhabditidae) with varied expression of the foraging gene, which encodes a guanosine 3,5'-monophosphate (cGMP)-dependent protein kinase (PKG). Thus, previous studies clearly indicated that cGMP-dependent PKG activation has many functions which influence different behaviors like locomotion and foraging. It was therefore assumed that this may affect the predation ability in *C. montrouzieri*. So, an attempt was made to explore the role of cGMP-dependent changes in the predatory performance of *C. montrouzieri*. The study was carried out at Indian Institute of Horticultural Research, Bangalore (12°58'N 77°35'E), Karnataka, India. Individual adult beetles were randomly selected from the established laboratory cultures and fed with different doses of cGMP analog viz., 8-Br-cGMP. The study clearly indicated involvement of cGMP in the predatory performance of *C. montrouzieri*. Further, the variation in the response to the administered cGMP dose was found to be performance linked, where good performers were found to be more responsive to cGMP treatment compared to corresponding poor performers in terms of quantity of mealybugs consumed. The change noticed in the predation levels of *C. montrouzieri* given cGMP treatment will be discussed in detail.

STUDIES ON BIOLOGY AND FEEDING POTENTIAL OF AN ANTHOCORID PREDATOR *CARDIASTETHUS EXIGUUS* POPPIUS (HEMIPTERA: ANTHOCORIDAE) ON *CORCYRA CEPHALONICA* STANTON,

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The present investigation aimed at studying biology and feeding potential of a potential anthocorid predator, *Cardiastethus exiguus* Poppius (Hemiptera: Anthocoridae) on the eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) under laboratory conditions to study the feasibility of the predator for mass production. To understand the effects of temperature variations on the life cycle of *C. exiguus*, the observations were taken from September through mid January. The results showed a mean hatching percentage of 82 percent under laboratory conditions. The total development period on an average was 21.23 days from September to early November. In the period from mid-November to mid-January development was prolonged, averaging 22.63 days as a result of low temperatures associated with the winter. Mean feeding potential throughout the nymphal period was 44.8 eggs from September to November. The increase in the nymphal development period was due to lower temperatures and resulted in the feeding potential changing to an average of 49.57 eggs, although the rate of egg consumption was quite steady. Longevity of adult males and females was, on average, 36.5 and 58.8 days, respectively and the commensurate mean feeding potential ranged from 185.7 eggs for males and 317.12 eggs for females throughout their life. Trials on host preference for the first time reported a very good preference of *C. exiguus* for eggs of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), with an average feeding potential of 58.5 eggs during the 20 day nymphal period. *Cardiastethus exiguus* was found to offer the better prospects for mass production.

OVIGENY IN SELECTED GENERALIST PREDATORS

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“Ovigeny” refers to the process of egg production in adult insects. “Pro-ovigenic” adult insects emerge with a fixed complement of mature eggs; whereas, “synovigenic” species continuously produce and develop eggs throughout adulthood. Very little work has been done on ovigeny in insect predators. We studied four predators, *Podisus maculiventris* Say (Heteroptera: Pentatomidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), *Delphastus catalinae* (Horn) (Coleoptera:

Coccinellidae), and *Geocoris punctipes* (Say) (Hemiptera: Geocoridae). We examined egg load at regular intervals (dependent on lifespan). Numbers of eggs laid were also recorded. In *P. maculiventris*, numbers of immature eggs increased with predator age whereas numbers of mature eggs declined providing clear indication of continuous egg production. Similar results were found in *G. punctipes*. In other predators, egg loads tended to increase with time, except in *D. catalinae*. However, numbers of eggs laid all increased with time. These findings suggest that egg production occurs during the adult stage in all these predators and that all predators studied were synovigenic to varying degrees.

GROUND-DWELLING PREDATORS IN CITRUS ORCHARDS IN SPAIN AND ITS PREDATORY ABILITY ON THE MEDITERRANEAN FRUIT FLY

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Ground-dwelling predators are widely acknowledged as providing valuable levels of regulation of pests. However, there is little information about their role on the control of some citrus pests which spend part of their life cycle in this habitat, such as the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemman) (Diptera: Tephritidae), one of the major pests in citrus orchards in Spain. We have evaluated the abundance and seasonal activity of the main ground-dwelling generalist predator groups in Spanish citrus orchards by using pitfall traps. A large number of generalist ground dwelling predators were found. Rove beetles (Coleoptera: Staphylinidae) were the most abundant-active group representing about 38.6% of the total number of predators collected, followed by spiders (Arachnida: Araneae) (28.9%), earwigs (Dermaptera) (18.0%), ground beetles (Coleoptera: Carabidae) (12.7%) and tiger beetles (Coleoptera: Cicindelidae) (1.8%). The wolf spider, *Pardosa cribata* Simon, (Areneae: Lycosidae) and the ground beetle, *Pseudophonus rufipes* (s.tr.) DeGeer (Coleoptera: Carabidae), were the most abundant predators of their respective groups. To assess the role of both predators in regulating *C. capitata* populations, we examined their functional response on third instars larvae, pupae and teneral-like adults under laboratory conditions. Additionally, *C. capitata* DNA species-specific primers have being designed to detect predation on this pest and tested for prey detection under laboratory and field conditions. Elucidating these trophic linkages is imperative if conservation biological control by native natural enemies is to be fully realized in successful citrus integrated pest management programs.

MOLECULAR ANALYSIS OF AN APHID-PARASITOID-PREDATOR FOOD WEB

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DNA-based methods of prey detection provide new insights into trophic interactions between pests and their natural enemies. In this study we utilized a DNA-based approach to resolve the complex trophic linking within an aphid-parasitoid-predator food web. Over 1,100 aphids and 1,200 invertebrate predators were collected at four dates between end of May and beginning of July 2005 in a winter wheat field near Warwick (UK). Aphids and predators were screened for DNA of the six most abundant aphid parasitoid species as well as for DNA of the English grain aphid, *Sitobion avenae* (Fab.) (Hemiptera: Aphidae), using multiplex PCR protocols. The application of this approach showed that although a high proportion of the predators (range 25-44%) had been feeding on aphids, also predation on parasitoids was substantial (range 9-16%). Semi-quantitative aphid-parasitoid-predator food webs were generated, depicting the species-specific trophic interactions within the community and their temporal changes. These webs indicated strong intraguild links between spiders and parasitoids, suggesting that aphid control by parasitoids is potentially restricted by specific generalist predators. These results highlight that intraguild interactions need to be considered at the food web level to further improve pest control strategies. Moreover, this study demonstrates that DNA-based methods can be used to construct complex quantitative food webs incorporating generalist predators.

NATURAL MORTALITY OF THE CALIFORNIA RED SCALE, *AONIDIELLA AURANTII*, IN CITRUS OF EASTERN SPAIN

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The management of the California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), a key pest in Spanish citrus, is mainly based on the use of insecticides. In recent years environmentally safer measures, as augmentative biological control by means of releases of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), are being encouraged. Knowledge on the role of the indigenous natural enemies that co-occur with the pest is an essential requisite before establishing any biological control programme. So far, the studies carried out in Spain have been limited to the quantification of parasitism, with values rarely reaching 30% at the end

of the season. Nevertheless, there are other natural sources of mortality, such as the host feeding produced by parasitoid females and the predation by generalist predators that remain unknown. In this work, the parasitism, host feeding and predation on *A. aurantii* have been quantified in three different citrus orchards located in Valencia (Eastern Spain). Furthermore, the species composition and relative abundance of parasitoids associated to *A. aurantii* has been determined.

SESSION 16

CAPACITY BUILDING THROUGH ACTION LEARNING IN REGION WIDE BIOLOGICAL CONTROL

A SURVEY OF NATURAL AND INTRODUCED PARASITOIDS OF THE OLIVE FRUIT FLY, *BACTROCERA OLEAE* (DIPTERA: TEPHRITIDAE) IN ISRAEL

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Olive cultivation occupies eight million hectares worldwide, with over ten million tons of olives annually (90% in the Mediterranean Basin). The olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) is a key pest of olive fruit, causing up to 50% in crop loss. Increasing biological control by natural enemies is a major goal of a regional effort to improve the yield, quality and income from olives in the eastern Mediterranean. For this project, a survey of natural enemies attacking *B. oleae* larvae in Israel was conducted during 2006-2008. Fruits were sampled whenever present at sites throughout the country, brought to the lab and held for emergence of flies and wasps, which were identified, sexed and counted. The total apparent parasitism rate over the survey was 11% (range 0-100%). Six taxa of parasitoid wasps emerged from olive fruits. The braconids *Psyttalia concolor* (Szépligeti) and *Diachasmimorpha kraussii* (Fullaway) (Hymenoptera: Braconidae) were the most abundant species. Not previously recorded from olive fly, *D. kraussii* was released during a biological control project against *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) from 2004-2007. Four species of Chalcidoidea were found only during the early season, before autumn. *Psyttalia concolor* had a wide geographic distribution, while *D. kraussii* was found in a narrower distribution, suggesting its origin in the planned releases. The sex ratio of *P. concolor* and *D. kraussii* was slightly male biased (55% and 70%, respectively). The data show that the activity of indigenous parasitoids is limited, which, together with the importation of new natural enemies, is the subject of further investigations.



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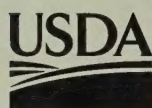


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